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PURPOSES

The main purpose of the Physiological Reviews is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology.

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BLOOD FIBRIN STUDIES

I. An Accurate Method for the Quantitative Analysis of Blood Fibrin in Small Amounts of Blood

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From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication July 19, 1921

Our present methods for the quantitative estimation of fibrin or fibrinogen are not satisfactory. The amount of blood required for single determination is large, so much so that determinations repeated at short intervals will at once introduce the factor of anemia. Many methods are relatively inaccurate and time consuming. Meek gives an excellent review of the development of these various methods (1).

The proposed method is quite satisfactory in our hands and enables a laboratory worker to estimate the fibrin content of the blood plasma accurately and with little expenditure of time. The amount of whole blood required is 8 to 10 cc. We estimate the *fibrin in whole blood* by determining the red cell hematocrit. Both values are given in all our experiments—the fibrin per 100 cc. plasma and per 100 cc. whole blood.

Previous workers have isolated fibrin or fibrinogen from the other blood elements in the following ways:

1. The blood sample was allowed to clot spontaneously. The fibrin was then freed from the other blood elements by grinding the clot and washing with water, alcohol and ether. The residue of fibrin was subsequently dried and weighed. The experimental error of this method is very large. It is practically impossible to wash the fibrin free from the other blood elements and in grinding and washing some of the fibrin may be lost. The amount of blood necessary for a relatively accurate analysis is large (50 to 100 cc.).

2. Spontaneous coagulation was inhibited by mixing the blood sample with a 3 per cent sodium fluoride solution. The formed elements were then allowed to settle, or were thrown down by the centrifuge. The fibrinogen was then precipitated from the plasma by the proper concentration of ammonium sulfate. This precipitate was washed thoroughly with water, alcohol and ether, then dried and weighed(2). This method is an improvement over the first in that the formed elements of the blood no longer complicate the analysis. The salting out process is not satisfactory, as it is well known that the various plasma proteins do not precipitate uniformly at a given salt concentration. Filtration of serum is a slow process and almost always involves a loss of material. Again, at least 50 cc. of blood must be used for a single analysis.

3. Spontaneous coagulation was inhibited by mixing the blood sample with sodium oxalate solution. The plasma obtained by centrifugalization was made faintly acid with 5 per cent acetic acid and heated for 10 to 15 minutes at 58° to 60°C. to precipitate the fibrinogen. The precipitate was separated by the centrifuge, washed, dried and weighed (3). The heat precipitation method of Whipple and Hurwitz (3) is also open to criticism. The heating may coagulate other of the blood proteins than fibrinogen, particularly if the acidity is not properly adjusted. It is known that a plasma very low in fibrinogen content may give little if any precipitate by this heat method—in other words, there is a serious error as we approach low values of blood fibrinogen. It seems evident from some experiments tabulated below, that this heat method is quite accurate when large quantities of plasma are used (25 cc.) and when the fibrinogen content of the blood is within normal limits.

4. Spontaneous coagulation was inhibited by mixing the blood sample with sodium oxalate solution. The plasma obtained by centrifugalization was diluted with 0.8 per cent sodium chloride solution and the fibrin coagulated by the addition of calcium chloride solution. A Kjeldahl determination on the washed fibrin gives the weight of fibrin in the sample of plasma used (4). This method developed by Cullen and Van Slyke (4) is a step toward greater accuracy. The dilution of the plasma permits of easy isolation of the fibrin. Ten cubic centimeters of plasma are sufficient for an analysis if the fibrin content of the blood is normal or above normal. If the fibrin content is below normal a larger amount should be used.

DEVELOPMENT OF THE METHOD. The blood was prevented from clotting by mixing with sodium oxalate solution. The centrifugalization was done in a graduated 15 cc. hematocrit tube in order that the per cent

In Volume LVIII, No. 3, January, 1922

In the paper on Blood Fibrin Studies, I, by Foster and Whipple, page 365, the authors have discovered errors in the table printed on page 367, which they desire to correct with the following statement:

"This table (table 1) contains values which are incorrect. It should be stated that a considerable excess of sodium oxalate will introduce an error in the gravimetric method as outlined in this paper. In all our experiments on fibrin values we used a minimum amount of oxalate solution to inhibit coagulation."—*Editor.*

Amer. J. of Physiology. 1922

of formed and fluid elements might be accurately determined. The analysis was made from a sample of plasma diluted with salt solution to facilitate the separation of the fibrin from the other blood proteins. The fibrin was coagulated by recalcification of the dilute mixture. The presence of the calcium oxalate formed by the addition of calcium chloride solution to the oxalated plasma made impossible the direct weighing of the dried fibrin thus obtained. The sample of fibrin was dried to a constant weight. The fibrin was then ignited in a crucible which was then reweighed to determine the amount burned off. The residue of calcium oxide was thus corrected for.

TABLE 1

Variation in amount of oxalate solution in this method; dog 17–160; condition, normal

SODIUM OXALATE 1 PER CENT SOLUTION	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
				Net	Average	100 cc. Plasma	100 cc Blood
cc.	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1.0	2 2	9.1	54	6.6 7.0	6.8	420	195
1.5	2 2	9.0	56	7.0 7.0	7.0	433	189
2.0	2 2	9.0	56	6.8 7.1	6.95	425	186
2.5	2 2	9.4	59	7.5 7.7	7.6	460	187
3.0	2 2	9.0	63	7.5 7.5	7.5	456	170

It seemed desirable to control a number of factors in this method.

1. We intended to use a minimum amount of *sodium oxalate*. The solution was usually 1.0 per cent, although it is recognized that this is slightly hypotonic. The use of 1 cc. of a 1.0 per cent solution with 9 cc. blood is close to the limit of safety. The addition of a little more blood to this mixture will often start blood coagulation. Table 1 shows the reaction due to increasing amounts of sodium oxalate solution (1.0 per cent or hypotonic.) The red cell hematocrit shows a progressive increase with increasing amounts of sodium oxalate solution per unit amounts of blood. The figures for fibrin per 100 cc. whole blood are

relatively constant up to the last dilution where the great excess of oxalate may have modified the blood coagulation.

2. It has been shown in this laboratory (6) that *centrifugalization* of oxalated dog's blood for 30 minutes at a speed of three thousand revolutions per minute will completely express all plasma from between the packed cells. This procedure will give uniform results and an accurate measure of the ratio of blood cells and plasma. From such observations it is easy to compute the relative amounts of red cells or plasma in per cent and the contained fibrin in plasma or whole blood. The separation is effected in calibrated 15 cc. centrifuge tubes graduated to $\frac{1}{10}$ of 1 cc.

TABLE 2

Dilution of clot by salt solution; dog 19–76; condition, normal

SODIUM CHLORIDE 0.8 PER CENT SOLUTION	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
				Net	Average	100 cc. Plasma	100 cc. Blood
cc.	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
40	2	9.2	59	8.2	8.15	514	213
40	2			8.1			
30	2	9.2	59	8.0	8.2	517	214
30	2			8.4			
20	2	9.2	59	8.4	8.3	524	217
20	2			8.2			

3. *Dilution of plasma to facilitate handling of clot.* Cullen and Van Slyke used an 0.8 per cent sodium chloride solution to dilute the plasma. This is a slightly hypotonic solution but when mixed with a small amount of 2.5 per cent calcium chloride solution the resulting mixture closely approaches blood isotonicity. We attempted a few plasma coagulations in distilled water plus calcium chloride solution and the result was a poorly formed amorphous mass. The 0.8 per cent solution plus the 2.5 per cent calcium chloride gave a uniformly good result and was used in all routine experiments.

Table 2 gives the results obtained with the same sample of blood using varying amounts of 0.8 per cent sodium chloride solution plus 2 cc. of 2.5 per cent calcium chloride solution. The smallest amount of salt solution which permits easy separation of fibrin from the other blood

proteins is the optimum amount because it permits easy handling of the sample. Too great dilution weakens the clot to such a degree that bits of fibrin are easily broken off and lost, while too scant a dilution does not make the texture of the clot coarse enough to permit the other blood proteins to be expressed easily and completely.

If 40 cc. of salt solution are used the clot formed from 2 cc. of plasma can be easily and safely manipulated with a glass rod and freed by gentle pressure from the enclosed fluids. Absolutely no part of the fibrin need be lost during this process and the resulting mass of fibrin can then be washed easily with water or salt solution. This simple procedure eliminated filtration which is tedious and often introduces inaccuracies.

4. Some experiments were performed to ascertain the extent of possible dilution. It was suspected that extreme dilution might inhibit the formation of fibrin in vitro under these conditions. We have no evidence that extreme dilution inhibits clot forming. Some experiments carried this dilution beyond ten times the customary dilution but the clot formed as usual. It was difficult to handle these very tenuous clots and errors from loss of material are to be expected under such condition.

Table 3 gives further control of the accuracy of this method and shows that considerable variation in the amount of the calcium chloride solution will not introduce an error. Excessive amounts of this salt may modify the coagulation. We use 2 cc. of a 2.5 per cent solution as a routine.

5. Coagulation does not take place at once on setting up a plasma mixture. Some experiments were done to determine the length of time necessary for complete coagulation, also the length of time the mixture may stand after coagulation without autolysis of the fibrin. It was found that the clotting time varied somewhat when the blood was abnormal. In some peptone injection experiments the blood clotted only after normal serum had been added to the mixture. Other experimental conditions to be reported in a later paper caused the same inhibition of coagulation.

Table 4 gives the results obtained by allowing samples of the same plasma to coagulate and stand at room temperature for different lengths of time. Before the half-hour period the clot was not sufficiently formed to permit manipulation. Uniform analyses were obtained from samples allowed to clot from one to twenty-four hours. After 48 hours the clot was generally autolysed and fragile. Occasionally samples have given normal readings after 48 hours. A clot should not stand more than 24 hours and the best results are obtained by expressing the fluid elements

from the clot after 2 or 3 hours. Cullen and Van Slyke recommend 10 to 15 minutes for the clotting of the recalcified plasma. In our experience this time is too short and may lead to error, at least in plasmata obtained from animals and abnormal human beings.

TABLE 3

Variations in the amounts of calcium chloride used; dog 19–45; condition, normal

CALCIUM CHLORIDE 2.5 PER CENT SOLUTION	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
				Net	Average	100 cc. Plasma	100 cc. Blood
cc.	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2	2	9.1	44	14.3	14.3	855	480
2	2			14.3			
3	2	9.1	44	14.6	14.6	873	489
3	2			14.6			
4	2	9.1	44	14.4	14.3	855	480
4	2			14.2			
5	2	9.1	44	13.8	14.1	843	473
5	2			14.4			
6	2	9.1	44	14.1	14.15	846	475
6	2			14.2			
7	2	9.1	44	14.1	14.15	846	475
7	2			14.2			
8	2	9.1	44	13.9	13.75	822	461
8	2			13.6			
9	2	9.1	44	13.0	13.3	79[illegible]	44[illegible]
9	2			13.6			
10	2	9.1	44	13.8	13.9	831	446
10	2			14.0			

In abnormal blood when clotting does not take place after recalcification coagulation will occur if 5 to 10 cc. of fresh serum are added. The usual method may be followed after that and good results obtained. In such samples a longer time for clotting must be allowed.

6. May oxalated blood stand for any length of time, and still be suitable for analysis of fibrin content? Table 5 shows the results of

experiments on the same sample of blood preserved 24 hours at room temperature and in the ice-box (3 to 7°C.). It is obvious that normal blood may stand either in the ice-box or at room temperature for 24 hours and still be suitable for a fibrin analysis. Bloods taken from cases of suspected liver injury should be analyzed at once as the tendency for autolysis in these cases is markedly increased. Our observations confirm those of Goodpasture (5) in reference to such cases.

TABLE 4

Optimum time for coagulation; dog 19–76; condition, normal

COAGULATION TIME	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
				Net	Average	100 cc. Plasma	100 cc. Blood
hours	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
½*							
1	2 2	9.2	59	8.2 7.8	8.0	505	209
1½	2 2	9.2	59	7.9 7.8	7.85	495	205
2	2 2	9.2	59	7.9 8.0	7.95	502	208
2½	2 2	9.2	59	8.0 8.4	8.2	517	214
24	2 2	9.2	59	8.0 8.0	8.0	505	209
48	2 2	9.2	59	†			

* Before 30 minutes clot too soft to handle.

† Clot autolysed.

Room temperature about 20°C.

7. As has been stated, the main objection to the existing methods for fibrin analysis was the fact that large quantities of blood were necessary for an accurate analysis. We wished to find the smallest amount of plasma that could be used and still give an accurate reading Cullen and Van Slyke (4) obtained uniform results with 10 cc. samples. We were interested to know whether smaller amounts could be used with accurate results. Accordingly, a number of analyses was made on the

same sample of blood using different amounts of plasma. Table 6 is a sample of a very large number of determinations made to establish this point. A 2 cc. sample of plasma is sufficient to give a reliable analysis by this method. Some experiments were carried out with a one cubic centimeter sample of plasma, but the results obtained were somewhat irregular.

8. The time necessary for complete dehydration of the clot in an oven at 110°C. varied with the quantity of fibrin. For the amount usually

TABLE 5

Comparison of fresh blood with blood preserved 24 hours; normal dogs

TIME	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		Remarks
				Net	Average	100 cc. Plasma	100 cc. Blood	
hours	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Fresh	2 2	10.0	50*	6.4 6.4	6.4	384	192	Dog 19–21 Dec. 30, 1919
24	2			6.5	6.5	390	195	Room temperature
Fresh	2 2	10.0	50*	5.0 5.4	5.2	312	156	Dog 18–60 June 24, 1918
24	2			5.2	5.2	312	156	Room temperature
Fresh	2 2	10.0	50*	6.4 6.6	6.5	390	195	Dog 17–28
24	2			6.5	6.5	390	195	Room temperature
24	2 2			6.5 6.9	6.7	402	201	Ice box 3°–7°C.

* Assumed value for hematocrit to give simple relative values for control and 24 hour specimens.

obtained from a 2 cc. sample of plasma, 3 hours were found to be sufficient. After this period constant weights were obtained for a given specimen. When larger amounts of plasma were used, a longer time was necessary. We encountered no specimens that were not thoroughly dry after 12 hours at 110°C. After weighing the crucible and fibrin contained therein the fibrin was ignited over a free flame. Samples were ignited and weighed at intervals from the time all the carbon had

disappeared up to one hour thereafter. A safe limit to place as the burning time is 15 minutes.

9. One essential control of the method was the serum coagulation in place of the routine coagulation by means of calcium chloride. The use of fresh serum obviates the formation of the calcium oxalate precipitate which is present in the usual reaction and insures a normal clot formation modified only by the presence of the diluent (0.8 sodium chloride) to insure a clot of loose texture. We may say that the results are identical whether the diluted plasma is coagulated by fresh serum or a calcium chloride solution (table 7).

TABLE 6

Comparative readings using varying amounts of plasma; dog 18–124; condition, normal

PLASMA	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
		Net	Average	100 cc. Plasma	100 cc. Blood
cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
10	43	54.2	54.0	638	362
10		53.8			
5		27.8			
5		27.5	27.72	655	371
5		27.9			
5		27.7			
2		11.1	11.1	656	372
2		11.1			

Dried serum was found to be unsuitable as a coagulant. At times, when dealing with abnormal blood, it is necessary to add serum to cause coagulation. If serum could be dried and kept on hand for this purpose, it would obviously be a convenience. Such serum does not act satisfactorily, however, and either fails to cause coagulation, or gives a muddy looking and fragile clot not suitable for analysis. Tissue extracts, which are sometimes used to furnish thromboplastin and promote coagulation, act much like dried serum and do not form a coagulum satisfactory for analysis. A fairly large amount of serum is necessary to cause complete coagulation of a sample of plasma. We found it necessary to add at least twice as much fresh serum as plasma to insure complete coagulations.

Control analyses were made on the same blood samples by the Kjeldahl method of Cullen and Van Slyke (4), the heat coagulation method of Whipple and Hurwitz (3) and the method here described to test the accuracy of results obtained by these methods and to find the limitations of each method. For our own satisfaction a great many analyses were made, but we will present here only three tables. It will be noted that

TABLE 7

Coagulation value of serum and calcium chloride compared; normal and abnormal dogs

CALCIUM CHLORIDE 1 PER CENT SOLUTION	FRESH SERUM	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		REMARKS
					Net	Average	100 cc. Plasma	100 cc. Blood	
cc.	*cc.*	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
									Dog 18–8
5		5			46.8	47.0	1100	693	High fibrin
5		5	9.2	37	47.2				Peritonitis
	15	5			46.7	46.45	1089	686	
	15	5			46.2				
2		2			17.9	18.35	1076	678	
2		2	9.2	37	18.8				
	5	2			18.3	18.35	1076	678	
	5	2			18.4				
									Pup 3 A
2		2			6.5	6.45	395	201	
2		2	8.85	49	6.4				
	5	2			6.8	7.1	435	222	
	5	2			7.4				
									Dog 19–21
5		5			31.8	31.6	807	315	
5		5	9.1	61	31.4				
	10	5			31.5	31.1	794	310	Fibrin above normal
	10	5			30.8				

the tables 8, 9 and 10 present blood samples of high fibrinogen content, normal and low fibrinogen content.

With normal blood (table 8) all of the estimations, except the 2 cc. samples by the Kjeldahl method, gave corresponding readings. The two cubic centimeter samples with the Kjeldahl give a reading that was high. It will be noted that this was true for all these experiments.

It will also be noted in table 9 (high fibrin content) that the result by the Kjeldahl and the dry weight methods checked within reasonable

limits. The heat coagulation method gave a result that was too high. This was probably due to the difficulty of washing such a large bulk of precipitate free from the other blood proteins. The Kjeldahl estimations on the 5 and 10 cc. samples were within the expected limits of accuracy but the estimation with 2 cc. of plasma gave a high reading. Both the 5 cc. and the 2 cc. samples by the dried weight method gave accurate readings.

TABLE 8

Comparative test of methods; normal dog; normal fibrin content

PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		REMARKS
			Net	Average	100 cc. Plasma	100 cc. Blood	
cc.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5	9.1	58	17.7	17.8	449	188	Dry weight method
5			17.9				
2	9.1	58	7.3	7.25	457	191	
2			7.2				
10	9.1	58		32.6	411	171.8	Kjeldahl method
10							
5	9.1	58		15.4	388	162	
5							
2	9.1	58		7.9	498	208.2	
2							
25	9.1	58	85.5	86.0	434	181.4	Heat coagulation method
25			86.5				

The greatest discrepancies between the three methods arose when a blood with a *low fibrin content* was analyzed (table 10). By the heat coagulation method not enough fibrinogen was precipitated to permit of analysis. The Kjeldahl method gave accurate results with 10 and 5 cc. amounts, but again gave a high reading with a 2 cc. amount of plasma. The dry weight method again gave uniform results with all amounts. From these experiments we were led to conclude that the dry weight method is more accurrate for small amounts of fibrin than the other methods described.

TECHNIQUE OF THE METHOD. Approximately 9 cc. of blood are collected from the vein by needle into a vaselined syringe and delivered immediately into a 15 cc. hematocrit tube which contains 1 cc. of a 1 per cent sodium oxalate solution. The tube with its contents is immediately inverted twice to insure thorough mixing. The mixture is centrifugalized at high speed (3000 revolutions per minute) for 30 minutes. The amount of cellular elements and plasma are read to tenths of a cubic centimeter. Exactly 2 cc. of the plasma are delivered by a calibrated

TABLE 9

Comparative test of methods; dog; distemper and pneumonia; high fibrin content

PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		REMARKS
			Net	Average	100 cc. Plasma	100 cc. Blood	
cc.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5 5	10.0	33	54.2 54.3	54.25	1246	835	Dry weight method
2 2	10.0	33	21.4 22.0	21.7	1246	835	
10 10	10.0	33		112	1287	858.3	Kjeldahl method
5 5	10.0	33		55	1231	821	
2 2	10.0	33		23.9	1373	919.9	
25 25	10.0	33	304.6 306.6	305.9	1406	942	Heat coagulation method

pipette into a tumbler containing 40 cc. of an 0.8 per cent solution of sodium chloride, and 2 cc. of a 2.5 per cent solution of calcium chloride. A similar duplicate sample is set up. The preparations are thoroughly mixed and allowed to stand at room temperature for 2 hours. To some abnormal blood plasmas it is necessary to add normal serum and a longer period may be required to insure complete coagulation.

The fibrin is freed from the fluid elements by gentle manipulation and pressure with a glass rod. The fibrin mass thus obtained is then washed in distilled water. The clear free fibrin is then placed in a small porce-

lain crucible and dried in an oven at 110°C. to a constant weight. This requires from 3 to 10 hours, depending on the amount of fibrin. After drying, the crucible is placed in a desiccator and allowed to cool. The weight of the crucible is recorded to tenths of a milligram. The fibrin is then ignited in the crucible over a Bunsen flame (15 minutes combustion time). While still warm the crucible is placed in a desiccator. When cool it is reweighed. The difference in the weights before and after burning is the weight of fibrin for the sample analyzed. Knowing

TABLE 10

Comparative test of methods; dog 18-87; chloroform poisoning; low fibrin content

PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		REMARKS
			Net	Average	100 cc. Plasma	100 cc. Blood	
cc.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5 5	9.2	47	4.0 4.0	4.0	92	49.0	Dry weight method
2 2	9.2	47	1.8 1.5	1.65	99	53	
10 10	9.2	47		7.28	87	46.4	Kjeldahl method
5 5	9.2	47		3.73	89	47.4	
2 2	9.2	47		2.42	145	77.3	
25 25	9.2	47			Mere trace		Heat coagulation method

the weight of fibrin per cubic centimeter of plasma and the ratio of red blood cells to plasma, we may calculate the amount of fibrin in 100 cc. of whole blood or blood plasma.

SUMMARY

A simple and accurate method for the quantitative analysis of blood fibrin is outlined. Blood plasma obtained from oxalated blood is coagulated slowly in a dilute solution and the delicate thin-meshed clot carefully washed, dried and weighed. Combustion and re-weighing of

residue gives the correct figures for the dry fibrin from which can be computed the plasma fibrin content. All determinations are made in duplicate. With accurate hematocrit readings the fibrin content of whole blood may then be estimated.

Various steps in this method are carefully controlled in the tabulated experiments and the optimum conditions are established.

Comparative determinations with other methods show interesting figures. The normal blood plasma may show practically identical figures in fibrin or fibrinogen analyses with all the common methods in use. This method is more accurate than the older methods when small amounts of plasma are used.

Abnormal plasma specimens may show greater differences in fibrin values and this method appears to be more accurate and dependable.

By this method it is possible to analyze blood samples at short intervals of time in a single experiment without introducing the factor of anemia (hemorrhage). The level of blood fibrin may thereby be established and plotted as a curve based on a large number of daily or even hourly observations. That this blood fibrin level may be influenced by a great number of factors will be shown in subsequent communications.

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BLOOD FIBRIN STUDIES

II. Normal Fibrin Values and the Influence of Diet

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Having available an accurate method for fibrin analysis using small amounts of blood we determined to study the normal fluctuations in blood fibrin which have been observed by other workers. By this method it is possible to make accurate fibrin determinations in duplicate using but 8 to 10 cc. of whole blood. It is therefore possible to make daily or even more frequent fibrin determinations in dogs without any complications due to anemia. As one studies the tables given below it is noted that the individual fluctuations in fibrin values are not as great as have been reported by other workers (1). However, under the most rigidly controlled conditions normal dogs show considerable fluctuation in fibrin values and we must look upon the fibrin of the blood plasma as a most labile substance. Compared with the other plasma proteins (2) which are so stable in total concentration the fibrin is truly mercurial in its changes. This may indicate a totally different source of production in the body as well as the greatest difference in general utility. The body under physiological conditions will not tolerate a low blood plasma fibrinogen content and promptly (a few hours) forms sufficient material to return the fibrinogen level to normal or above normal.

Experimental observations. Dogs were used in all these experiments and unless otherwise stated were in excellent physical condition. The daily samples were taken before the food or diet mixture was given. Dogs were fed once daily unless note is made to the contrary. The fibrin method has been fully described in the first paper of this series. All determinations were done in duplicate. An important control experiment is found in table 14, which shows the minimal action which follows daily bleedings. This large dog was bled 40 cc.

every day over a long period with little disturbance of the fibrin level. Smaller bleedings have even less influence on the curve of fibrin values. We feel confident therefore that our method of removing small samples of blood from normal dogs does not in itself modify the curve of fibrin values as determined by subsequent analyses.

The blood fibrin values for normal dogs are given in tables 11, 12 and 13. The average figures for mixed diet are close to 400 mgm. fibrin per 100 cc. plasma. The red cell hematocrit values for normal dogs are slightly less than half the plasma figures.

TABLE 11

Blood fibrin of normal dogs; liberal mixed diet

DATE	DOG		WEIGHT	FIBRIN	
	Number	Type		100 cc. Plasma	100 cc. Blood
			pounds	*mgm.*	*mgm.*
5/16/19	19–104	Pup	17	506	248
12/21/18	15–56	Bull	47	310	140
9/11/18	19–2	Bull	26	351	161
9/11/18	19–21	Pointer	40	306	138
9/11/18	19–1	Bull	40	372	192
12/ 9/18	19–66	Terrier	22	344	150
6/24/19	19–68	Spitz	15	453	226
6/27/19	19–93	Bull	25	462	271
1/22/19	18–30	Bull	35	314	140
12/27/19	16–52	Spitz	20	403	201
6/13/18	18–103	Bull	35	430	159
9/ 5/18	19–124	Mongrel	40	457	228
	18–38	Coach	30	364	182
Maximum				506	248
Minimum				306	140
Variation				200	108
Average				390	187

Fasting conditions give more stability to the blood fibrin values and the physiological fluctuations are less. The average fibrin values are less in fasting than during periods of mixed diet. "Mixed diet" in these papers means a liberal diet of table scraps obtained from the University Hospital, consisting of cooked meat, bones, bread, potatoes, rice, macaroni and vegetables. In certain cases there may be a considerable excess of food given which permits individual selection of food. One dog may prefer meat, or bones or potatoes, etc. The importance of this factor will come out in some later experiments.

It has been stated elsewhere (1) that individual dogs showed less variations in their fibrin values but between different normal dogs the difference in fibrin values might be very considerable. Under uniform conditions we feel that a normal adult dog will not show great fluctuations in blood fibrin values (table 13). It may be safe to say that the physiological variations in the curve of blood fibrin under uniform conditions should not exceed 20 to 30 per cent. Eventually we may hope to understand the factors which cause even these small fluctuations in the blood fibrin values.

TABLE 12

Blood fibrin of normal fasting dogs; food withheld as indicated—water in cage

DATE	DOG		WEIGHT	FIBRIN		REMARKS
	Number	Type		100 cc. Plasma	100 cc. Blood	
			pounds	*mgm.*	*mgm.*	
5/19/19	19–104	Bull	17	376	181	After 3 day fast
5/19/19	17–157	Coach	21	401	219	After 3 day fast
9/23/18	19–2	Bull	26	303	145	After 12 day fast
9/23/18	19–21	Pointer	40	334	164	After 3 day fast
9/23/18	19–1	Bull	40	412	218	After 3 day fast
1/29/19	19–78	Airedale	26	344	130	After 2 day fast
2/ 4/19	19-85	Mongrel	24	398	121	After 2 day fast
2/11/19	19-50	Mongrel		349	184	After 2 day fast
2/25/19	19–6	Mongrel	30	388	152	After 3 day fast
6/30/19	19–124	Mongrel	40	354	157	After 3 day fast
Maximum				412	219	
Minimum				282	121	
Variation				130	98	
Average				358	167	

An important factor is controlled in table 14. Daily bleedings of 40 cc. whole blood removed by hypodermic needle from the jugular vein does not materially disturb the fibrin level in this dog. Presumably as small a dog as ten pounds should tolerate a daily bleeding of 10 cc. without showing serious disturbance of the fibrin or hematocrit values. This dog (table 14) does show a loss in red cell hematocrit per cent but it may be recalled that this rice diet is not favorable for hemoglobin regeneration (3). The general condition remained excellent throughout the long experiment. There is a slight tendency for the fibrin values to rise during the week following the initiation of the

daily bleedings. With considerable hemorrhage, this tendency may become quite marked and may carry the fibrin values much above the normal level.

The experiments given in table 15 are complicated by a number of factors but they give a very important lead which is followed out in other experiments. We note in passing that some of these data have been reported elsewhere in connection with experiments dealing with liver necrosis and repair as influenced by dietary and other factors (4), (5). Three different group experiments are included in this table. Each group of experiments was performed upon a litter of pups under approximately identical conditions. The only variable was the special

TABLE 13

Individual fluctuations of blood fibrin under standard conditions; dog 15–56; white bull, male; mixed diet

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
12/21/18	10.1	55	5.1	310	140	
1/ 6/19	9.7	56	4.6	278	130	Weight 47 lbs.
1/13/19	10.4	58	4.9	300	127	
1/20/19	9.2	54	4.7	291	134	
1/24/19	9.0	53	5.5	340	159	
1/27/19	9.3	53	4.7	288	137	
2/ 4/19	8.8	51	4.75	292	143	Weight 46.2 lbs.
Maximum				340	159	
Minimum				278	130	
Variation				62	29	

diet for a period of four days preceding the unit chloroform anesthesia of one hour. All pups were killed with ether 48 hours after the chloroform anesthesia. The blood fibrin was determined at autopsy and the extent of liver necrosis and fatty degeneration estimated from histological sections of the liver.

When a severe liver necrosis is present we expect to find low fibrin values and some of these pups show this reaction but not to an extreme degree. When a slight liver injury is found we may observe a rise above normal in fibrin values. This may be explained in part as a stimulating or irritative reaction similar to the abscess reaction given in later experiments (paper IV). But we note further that the pups

fed meat and liver show remarkably high fibrin values as compared with carbohydrate feeding. We believed other factors besides liver injury were concerned and decided to test out various limited diets to ascertain whether or not such diets could influence the level of blood fibrin values in normal dogs. It seemed possible to us that the general stimulating action which protein feeding exerts upon body

TABLE 14

Blood fibrin as influenced by daily bleedings; bled 40 cc. and fed 1 pound rice each day; dog 19–21; adult female pointer

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
2/10	9.0	51	4.90	334	171	After 3 day fast Weight 37 lbs.
2/19	9.5	49	6.70	394	199	Rice diet for 9 days
2/19	Dog bled 40 cc. per day subsequently					
2/21	9.9	43	6.20	365	207	
2/23	8.6	46	6.85	416	225	
2/26	9.1	42	6.90	410	239	Normal Weight 37 lbs.
2/28	10.1	45	6.20	365	203	Weight 37.7 lbs.
3/ 4	9.5	43	5.80	342	200	Weight 37.7 lbs.
3/ 7	10.6	46	6.35	375	202	Weight 37.3 lbs.
3/11	10.1	45	5.90	347	193	Weight 40 lbs.
3/17	10.0	44	6.45	380	213	Weight 39.2 lbs.
3/19	9.1	46	6.60	397	216	Weight 38.5 lbs.
3/25	9.2	41	5.70	337	198	Weight 38 lbs.
3/29	10.3	44	6.30	336	206	Weight 41 lbs.
4/ 2	10.0	42	5.60	346	201	Weight 42 lbs. Normal Bleeding discontinued
Maximum				416	239	
Minimum				334	171	
Variation				82	58	

metabolism might be concerned in the higher level of blood fibrin which may obtain on a meat diet. It is clear that this factor alone will not explain all the reactions noted below, particularly the fact that different proteins of animal origin have very different stimulating effects upon the blood fibrin values. Compare also the low fibrin values with the thyroid feeding (table 22).

TABLE 15

Blood fibrin levels influenced by diet factors plus chloroform injury; four days special diet followed by one hour chloroform anesthesia; pups of 4–10 weeks of age—three different litters

DOG NUMBER	WEIGHT	DIET	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			LIVER		REMARKS
					Average	100 cc. Plasma	100 cc. Blood	Necrotic	Fat	
	lbs.		*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	
3A	5.0	Milk	8.8	49	6.45	395	201	0	20	Skim milk
3C		Carbohydrate	9.3	38	4.85	284	177	0	33	Potatoes
3D		Meat	9.3	46	9.50	569	309	20	66	Cooked skeleta' muscle
3E		Fat	9.3	44	2.65	157	89	25	80	Lard, butter and oil
3F	5.6	Liver	9.1	38	17.00	1000	621	0	tr.	Cooked liver
4A	6.4	Gelatin	9.2	52	1.55	95	45	40	tr.	25 grams gelatin (stomach tube)
4B	6.0	Fat	8.8	48	1.50	91	48	80	tr.	Beef fat and crackermeal
4C		Fat and carbohydrate	9.1	39	2.60	150	91	35	20	
4E	5.2	Carbohydrate	9.1	46	5.20	313	169	0	tr.	Potatoes, rice, crackermeal and sugar
4F	3.4	Meat	9.5	38	11.10	657	408	tr.	50	Cooked skeletal muscle
5A	6.2	Gelatin and sugar	9.5	41	2.85	162	96	tr.	50	25 grams gelatin and 25 grams cane sugar
5B	6.0	Beef heart	9.1	43	1.40	83	48	50	20	Cooked beef heart
5C	6.2	Fat and carbohydrate	9.5	48	2.75	162	85	75	20	Butter, lard, potatoes
5D	5.7	Liver	9.2	44	5.45	324	183	0	75	Cooked liver
5E	4.4	Meat	9.5	37	2.85	166	104	50	20	Cooked skeletal muscle

A considerable number of observations have established the fact that for a given dog the fasting value for fibrin is comparatively stable. The fasting value for blood fibrin is established with considerable uniformity by a fasting period of 3 days, regardless of the diet given preceding the fast. We note alternate periods of fasting and limited diet feeding in three parallel experiments (tables 16, 17 and 18). The sequence of diets is the same in all dogs and the duration of the experiments is also identical. It is possible that a change of this sequence might modify the reaction. But it seems pretty clear that the heavy animal protein feeding as a rule gives higher fibrin values. The changes are not great but they are pretty uniform and exceed any fluctuations noted during fasting periods. The very young dog, the adult and the old dog all react much alike.

A remarkable reaction due to an *unusual diet factor* is recorded in tables 19 and 20. Cooked pig stomach fed in liberal amounts seems to be responsible for lifting the blood fibrin level almost 100 per cent above the fasting level. This high level is well sustained (table 19) for a week on this unusual diet and promptly subsides to the normal level when this food factor is withdrawn. The pig stomachs were received fresh, were then washed, cut up and cooked thoroughly in boiling water. The water was poured off and this material was then fed in the amounts indicated. This food was eaten with relish by both dogs.

The second experiment records the prompt reaction which is observed in the blood fibrin level following a large feeding of cooked pig stomach. We are unable to state what factors in this diet may be responsible for this truly remarkable reaction. One should first test the intestinal epithelium and the smooth muscle, but the necessary experiments are not yet at hand. The reaction to liver epithelium may indicate that gland cells are particularly concerned in this peculiar diet reaction.

Pregnancy may be classed as a physiological reaction and it is of interest to record the findings during normal pregnancy in a dog (table 21). The normal level during the last two weeks of gestation is fairly constant in view of the fact that the dog had a very liberal mixed diet. The uniform fall of the hematocrit during this time is of considerable interest but need not be discussed here except to note its importance in explaining the rise in the fibrin values for whole blood. It is quite remarkable that there should be a distinct fall in fibrin values in the two to three days following birth of the pups. There is a noticeable rise above normal in fibrin values on the seventh to tenth days after

TABLE 16

Blood fibrin influenced by diet factors; normal pup 19–2

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
9/13	25.2	9.1	57	5.30	333	143	Analysis after 3 day fast
9/26	23.7	10.3	53	4.60	277	129	Fasted 12 days
9/26	Diet: Rice, crackermeal and sugar, 100 calories per kilo						
9/27		10.0	50	4.70	282	141	
9/28		8.9	57	5.30	358	155	
9/29	23.3	9.2	49	4.85	294	149	
	3 days' fast						
10/ 1	22.0	9.0	52	4.80	295	141	
10/ 1	Diet: Lard and butter mixed with crackermeal and weak soup, 100 calories per kilo						
10/ 2	22.1	9.8	45	4.85	287	159	
10/ 4	22.0	8.6	51	4.70	291	142	
10/ 6	22.1	8.7	48	3.70	226	117	
	3 days' fast						
10/ 8	Diet: Milk and crackermeal, 100 calories per kilo						
10/ 9	21.0	9.3	54	5.65	348	161	
10/11	21.8	9.5	47	5.70	342	180	
	3 days' fast						
10/17	Diet: Beef heart, 100 calories per kilo						
10/18	20.8	9.4	52	5.30	317	152	
10/21	21.5	9.6	52	6.35	386	185	
	3 days' fast						
10/24	Diet: Cooked pig's or beef liver, 100 calories per kilo						
10/25	21.0	9.6	54	5.05	309	142	
10/26	20.4	9.4	60	7.60	480	194	
10/27	20.6	9.8	57	7.10	448	195	Dog active

TABLE 17

Blood fibrin influenced by diet factors; normal adult dog 19–21

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
9/13	38.6	9.7	54	4.95	302	139	Analysis after 3 day fast
9/26	35.5	10.0	61	5.00	314	122	Fasted 12 days
9/26	Diet: Rice, crackermeal and sugar, 100 calories per kilo						
9/26		10.3	57	7.40	454	194	
9/27		11.1	53	6.90	398	229	
9/29	36.9	9.6	49	7.30	439	224	
	3 days' fast						
10/ 1	35.0	9.4	51	6.75	411	201	
10/ 1	Diet: Lard and butter mixed with crackermeal and weak soup, 100 calories per kilo						
10/ 2	35.5	7.7	51	7.30	330	173	
10/ 3	35.2	10.5	47	5.00	300	157	
10/ 6	35.3	10.6	52	6.20	371	179	
	3 days' fast						
10/ 8	Diet: Milk and crackermeal, 100 calories per kilo						
10/ 9	35.6	9.7	52	6.50	393	191	
10/11	35.8	9.5	46	6.60	394	214	
	3 days' fast						
10/17	Diet: Beef heart, 100 calories per kilo						
10/18	33.2	10.0	49	7.20	430	220	
10/21	33.8	9.6	49	7.00	421	215	
	3 days' fast						
10/24	Diet: Cooked pig's or beef liver, 100 calories per kilo						
10/25	32.3	9.6	47	5.25	314	167	Food not eaten well
10/26	33.0	9.8	50	6.00	361	180	
10/27	32.6	9.5	51	5.85	354	175	

TABLE 18

Blood fibrin influenced by diet factors; normal old dog 19–1

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
9/13	39.0	9.4	46	6.15	368	198	Analysis after 3 day fast
9/26	34.8	10.9	50	6.40	391	196	Fasted 12 days
9/26	Diet: Rice, crackermeal and sugar, 100 calories per kilo						
9/27	36.3	10.3	44	5.90	345	194	
9/28	35.8	9.6	42	6.20	365	213	
9/29	37.3	9.9	45	6.90	408	185	
	3 days' fast						
10/ 1	35.0	9.6	47	7.00	418	222	
10/ 1	Diet: Lard and butter mixed with crackermeal and weak soup, 100 calories per kilo						
10/ 2	35.2	9.0	49	8.00	408	209	
10/ 3	34.9	9.7	48	5.70	342	179	
10/ 6	35.4	10.1	44	8.70	512	285	
	3 days' fast						
10/ 8	Diet: Milk and crackermeal, 100 calories per kilo						
10/ 9	34.8	9.9	46	9.80	604	324	
10/11	35.8	10.3	44	8.55	501	283	
	3 days' fast						
10/17	Diet: Beef heart, 100 calories per kilo						
10/18	33.6	9.9	46	8.80	523	280	
10/21	34.7	9.9	48	8.70	520	268	
	3 days' fast						
10/24	Diet: Cooked pig's or beef liver, 100 calories per kilo						
52/01	34.0	9.7	48	8.65	519	268	
10/26	34.2	9.9	54	8.60	523	243	
10/27	34.0	9.3	49	8.75	530	268	

TABLE 19

Blood fibrin influenced by diet of cooked pig stomach; dog 19–68; spitz, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
4/ 3	16.0	10.1	53	5.80	350	166	After 3 day fast
4/ 5	Fed cooked pig stomach—1 lb. daily						
4/ 5	14.6	10.0	49	5.30	308	157	
4/ 7		10.1	47	5.90	350	186	
4/ 8		10.0	46	10.00	592	320	
4/ 9		9.9	44	12.90	762	424	
4/10	15.5	9.2	41	11.50	681	400	
4/11		10.1	38	10.30	612	382	
4/14	15.4	9.5	46	10.60	634	340	Normal
	Feeding of cooked pig stomach discontinued						
4/16		9.3	46	6.60	396	213	
4/18	14.6	9.6	46	5.60	334	183	

TABLE 20

Blood fibin influenced by diet of cooked pig stomach; dog 19–124; mongrel, male, weight 30 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
7/23	10.3	59	5.70	353	144	After 3 day fast
10 a.m.	Fed 2 lbs. cooked pig stomach					
11 a.m.	10.0	57	6.00	370	159	
12 m.	10.8	59	6.35	390	161	
1 p.m.	8.4	57	6.10	390	167	
1 p.m.	Fed 2 lbs. cooked pig stomach					
3 p.m.	10.3	55	8.40	512	228	
4 p.m.	10.6	54	8.60	518	238	
7/24	9.7	58	7.00	435	182	
10 a.m.	Fed 2 lbs. cooked pig stomach					
12 m.	7.6	51	5.85	285	143	
2 p.m.	10.5	49	6.40	379	195	
7/25	6.9	47	8.40	534	285	
7/26	5.2	44	7.00	470	262	
7/28	8.9	54	6.80	423	195	

delivery. It seems best to explain this rise as due in part at least to the reaction at the placental sites. This is a reaction similar in many respects to the reaction taking place about injured tissue anywhere in the body (aseptic abscess, peritonitis, etc.). Such reactions if uncomplicated always cause distinct increase in the blood fibrin values (refer to table 36 below).

TABLE 21

Blood fibrin during pregnancy; dog 18–40; adult mongrel; liberal mixed diet

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
12/27	9.0		6.60	403		Weight 30.8 lbs.
12/30	9.4	48	5.75	346	181	
1/ 2	8.5	44	6.55	395	223	Weight 31.8 lbs.
1/ 4	9.5	41	6.90	409	240	
1/ 6	10.4	38	7.80	450	277	
1/10	10.1	37	6.60	381	242	
1/13	Pups born in early morning—slight loss of blood					
1/13	10.0	36	4.55	262	168	
1/15	10.2	38	5.20	300	185	Pups nursing. Dog in good condition
1/20	9.3	41	8.30	490	290	
1/22	10.2	40	8.90	517	310	Weight 25.2 lbs.
1/24	10.3	42	8.80	521	303	
1/27	9.5	41	6.50	383	226	
2/ 4	9.5	42	6.60	390	226	
2/10	8.2	41	7.80	478	281	
2/17	8.7	45	8.20	395	208	
2/24	8.9	49	6.15	376	190	Pups normal and nursing
3/ 5	9.0	50	6.40	391	196	Weight 23.2 lbs.
3/11	9.8	53	7.30	444	209	Excellent condition
3/19	10.1	49	6.20	384	160	Nursing pups

Thyroid feeding in small amounts may give a reaction properly called physiological but larger amounts may cause reactions classed as pathological. The amount of thyroid fed in this experiment may be considered large but we wished to give enough of the dried gland to cause loss of weight. With this reaction we feel confident that there was a distinct increase in the daily output of urinary nitrogen, increased endogenous metabolism and consequent breaking down of tissue pro-

tein. How will this reaction compare with the inflammatory reaction? In both instances we have a decided increase in tissue katabolism. Inflammation whether septic or aseptic will give a rise in the blood fibrin level but we note no such reaction following thyroid feeding (table 22). There seems to be a gradual lowering of the blood fibrin level as the loss of body weight due to thyroid feeding progresses. This type of tissue katabolism does not call out a reaction on the part of the fibrin similar to that constantly observed in association with the tissue katabolism of inflammation or cell necrosis.

TABLE 22

Blood fibrin influenced by thyroid feeding; dog 19–21

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
7/ 5	37.00	9.8	44	8.10	522	291	
7/ 7	Fed cooked rice—1 lb. daily						
7/ 7		9.9	43	7.60	480	274	
7/ 9		10.4	44	7.70	451	252	
7/10	Fed 1 lb. cooked rice and 1 gm. desiccated thyroid (Armour) daily						
7/10		10.1	39	7.70	448	273	Normal
7/12	35.00	10.1	40	6.00	350	210	
7/14	34.75	9.9	46	7.60	450	245	
7/16		9.5	47	7.40	433	233	
7/18	33.75	9.9	44	6.10	360	203	
7/19	32.00	10.5	45	5.90	346	191	Normal

SUMMARY

Fibrin is a blood plasma protein which is very labile and its fluctuations are due to a variety of stimuli. Many of these stimuli have been recognized.

Our experiments indicate that *diet substances* may be concerned in these fluctuations of fibrin values.

Individual dogs under uniform conditions have a relatively constant fibrin level. Fluctuations should not exceed 20 to 25 per cent.

Fasting dogs have individual blood fibrin levels which are relatively constant. This level is usually established by a 3-day fast.

Normal blood plasma fibrin values should fall within the limits of 250 to 500 mgm. per 100 cc. plasma. The figures for whole blood should always be given as this is the *efficient fibrin level* which concerns hemorrhage. In normal dogs this figure is a little less than one-half the plasma values as the red cell hematocrit is usually 50 to 57 per cent.

Diets rich in animal protein (meat, liver, etc.) favor a high blood fibrin level as contrasted with fasting, fat or carbohydrate feeding.

A striking reaction is noted in association with a diet of cooked pig stomach. This diet causes a rise in the blood fibrin level which may be an increase of 100 per cent over the fasting level.

Pregnancy may show fluctuations in the blood fibrin values which are difficult to explain.

Thyroid feeding sufficient to cause loss of weight may be associated with a slowly decreasing level of blood fibrin.

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BLOOD FIBRIN STUDIES

III. Fibrin Values Influenced by Transfusion, Hemorrhage, Plasma Depletion and Blood Pressure Changes

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At various times in this laboratory the properties of the blood serum proteins have been investigated. The remarkable *stability* and *stabilizing value* of the *blood serum proteins* have been established by these experiments (1). Usually the blood plasma protein (fibrin or fibrinogen) was followed in these same experiments. Also the investigation of many problems related to the circulating blood volume has enabled us to make fibrin determinations in experiments of the type recorded in this paper without duplication of effort. Some of these studies in blood volume have been published (2) and others will follow in the near future.

Fibrin is a very labile substance when compared with the other blood proteins. Sudden changes in blood volume may be brought about by transfusion, hemorrhage or any combination of transfusion and hemorrhage. The blood fibrin concentration is thereby modified. What reaction is to be expected and how does the fibrin reaction compare or contrast with the reaction of the stable serum proteins? Will sudden and extreme changes in blood pressure cause any reaction in the blood fibrin values? The experimental data given below furnish the answer to these questions.

It may be recalled that experiments done in this laboratory (3) give evidence that the level of blood serum proteins is a pretty fixed constant for any given animal. This is in striking constrast to the fibrin (a plasma globulin) which is influenced by diet factors and many other stimuli which have little or no influence upon the serum proteins. Published experiments (4) show how rapidly (24 hours) the blood fibrin is regenerated after its washing out by plasma depletion, but under simi-

lar conditions the serum proteins can be brought back to normal only after a time interval of several days. The site of production of fibrinogen may be the liver aided perhaps by a reserve in other tissues (5). The site of production of the serum proteins may also be the liver but the mechanism seems to be very different. It is a simple matter for the body to form large amounts of fibrin and maintain or raise its blood plasma fibrin level. But it is a difficult matter for the body to form or regenerate the other blood serum proteins when these substances are removed or diluted. This may indicate that the fibrin is being constantly used up and manufactured anew in the body The same reasoning may suggest that the serum proteins are not rapidly used in the body economy. That they are essential to the normal environment of the body cells has been amply substantiated (1).

What evidence can be brought forward to indicate a *constant use and consumption of fibrin in the body?* When the blood will not coagulate in contact with tissues because of lack of fibrinogen or other abnormality, we at once observe in man or animal bleeding about the teeth and in some or all parts of the intestinal tract. This indicates that small injuries in the mouth cavity, especially about the teeth, and in the stomach or intestine are constantly present and are sufficient to rupture capillary vessels. Normally these breaks are repaired at once by the use of fibrin as the body of the clot. Probably all types of pressure or slight injury to the surface of the body may break the capillary wall and require fibrin to seal these breaks. We are familiar with the large black and blue areas which follow trivial bruises in patients whose blood does not clot normally in contact with tissues. All types of inflammation and cell injury stimulate fibrin production and probably are associated with a deposition of fibrin and so its withdrawal from the blood plasma. All these facts point to a rapid production and consumption of fibrinogen in the body.

Experimental observations. The greater part of these experiments was done with the primary object of investigating changes in the volume of the circulating blood. Many of these observations have been published (2). As a part of this work blood samples were taken both for fibrin estimation and for blood volume determinations at intervals as stated in the tables. Solutions of inert red dyes (vital red group (2)) were likewise introduced as a part of the blood volume estimation. The influence of these procedures upon the fibrin values is shown in a control experiment in which the usual dye injections and blood sample removals were performed at the usual time intervals *without* any injec-

tion, transfusion or hemorrhage. The control experiment (table 27) shows a fibrin level which remains practically unchanged throughout the period of observation. The slight increase after 24 hours is probably due to the bleedings of the preceding day.

All injections were made by hypodermic needle inserted in the jugular vein. No anesthetic was required. Three experiments (tables 30 and 31) were performed under ether anesthesia as cannulae were placed in both artery and vein. This technique has been described elsewhere.

TABLE 23

Intravenous injection of Locke's solution

Dog 18–39. Mongrel. Male. Weight 27 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			PLASMA VOLUME	REMARKS
		Average	100 cc. Plasma	100 cc. Blood		
	per cent	*mgm*	*mgm.*	*mgm.*	*cc.*	
July 11	55	6.7	393	179	479	
Rapid injection into jugular vein—600 cc. Locke's solution						
July 11	45	5.5	315	172	685	Immediately after
July 11	45	5.0	294	161	474	3 hours after
July 12	50	9.9	662	335	493	
July 13	42	8.3	500	291		
Dog 19–99. Bull dog. Male. Weight 33 lbs.						
July 11	36	9.3	600	384	886	
Rapid injection into jugular vein—750 cc. Locke's solution						
July 11	33	8.3	520	351	1195	Immediately after
July 11	31	9.2	574	396	1145	3 hours after
July 12	35	10.8	704	460	790	
July 13	35	8.5	542	361		

Table 23 presents the data from two experiments in which large amounts of Locke's solution were given rapidly intravenously. These experiments are to be compared with subsequent experiments in which acacia solutions or serum are substituted for the Locke's solution. We note a distinct difference. Following the injection of Locke's solution there is a fibrin reaction not observed after the injection of acacia. Therefore the simple dilution of the plasma fibrinogen values is not responsible. It appears more probable that the very presence of these

salts (Locke's solution) in unusual concentration in the plasma is responsible for the rise in fibrin values observed after such injections.

Table 23 therefore shows a positive wave of increase of blood fibrin on the day following the injection of Locke's solution. In the second experiment, with an abnormally high fibrin value, the level is further increased on the next day following the injection. This rise is temporary and the return toward normal is evident on the second day following the injection (refer also to table 31). The blood volume figures are given but will not be discussed at this time. These figures indicate a very rapid escape of the Locke's solution from the blood circulation.

TABLE 24

Intravenous injection of acacia solution (6 per cent)

Dog 18–123

DATE	B. B. C. HEMATOCRIT	FIBRIN			PLASMA VOLUME	REMARKS
		Average	100 cc. Plasma	100 cc. Blood		
	per cent	*mgm.*	*mgm.*	*mgm.*	*cc.*	
August 8	53	6.6	480	210	1063	
Intravenous injection—520 cc. of 6 per cent acacia solut on						
August 8	39	3.4	219	133	1613	5 minutes after injection
August 8	37	3.8	248	157	1240	3 hours after injection
August 9	43	7.0	459	262	1068	
Dog 17–38						
August 8	58	5.9	456	194	1416	
Intravenous injection—635 cc. of 6 per cent acacia solution						
August 8	41	2.6	168	59		5 minutes after injection
August 8	40	3.2	205	122	1231	3 hours after injection
August 9	45	6.1	398	221	1315	

Table 24 shows two experiments in which acacia solutions 6 per cent were given intravenously in amounts approximately equal to the determined plasma volume. It is known that acacia solutions disappear slowly from the blood stream (6),(7). Therefore the dilution of the fibrin values would be more lasting than following the Locke's solution injections. The fibrin reaction to this dilution is practically nil and we see a slow return to normal which may well parallel the removal of the acacia from the blood plasma. The behavior of the blood volume and

hematocrit figures are of great interest but cannot be discussed at this time. It should be kept in mind that each determination means a considerable loss of blood (50 to 60 cc.) as several analyses were made in each period and this explains some of the hematocrit fluctuations.

Table 25 shows again the same general type of reaction noted in the preceding table. Smaller amounts of acacia solutions are injected. These experiments illustrate an interesting reaction which may lead to serious errors in fibrin determination. Blood removed immediately

TABLE 25

Intravenous injection of acacia solution (6 per cent)

Dog 19–126. Mongrel. Male. Weight 39 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
April 8	49	10.7	708	363	Blood volume 1867 cc.
Intravenous injection—590 cc. of 6 per cent acacia solution					
April 8	37	5.8	377	216	After injection, coagulation only on adding serum
April 8	43	6.4	407	233	3 hours later
April 9	44	9.5	354	199	
Dog 18–4. Mongrel. Female. Weight 42 lbs.					
April 9	60	7.2	522	208	Blood volume 1962 cc.
Intravenous injection—630 cc. of 6 per cent acacia solution					
April 9	53	3.8	259	121	After injection, coagulation only on adding serum
April 9	52	4.6	319	154	3 hours later
April 10	50	6.6	430	214	

after acacia injection often does not clot and normal coagulation is only obtained by the use of serum. At times this antithrombin reaction will be less definite and may only *delay* blood coagulation, rendering it incomplete for many hours. If this is not controlled, incomplete fibrin values may be returned.

Table 26 also gives data on acacia injection. Smaller amounts of concentrated (20 per cent) acacia solution were injected. One experiment shows an absolutely negative reaction although the initial value for

blood fibrin is abnormally high. The second experiment shows immediate fluctuation and a slight increase on the second day.

Table 27 is of considerable interest as it gives control of all the factors concerned in the preceding experiments—that is, dye injection and blood sample removal. The procedure is identical in every respect but for the fact that no injection of acacia or Locke's solution is made. There are slight fluctuations during the period of observation but the fibrin values are relatively constant. The slight rise on the second day

TABLE 26

Intravenous injection of concentrated acacia solution (20 per cent)

Dog 18–24. Mongrel. Weight 30 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
March 12	61	10.95	815	315	Blood volume 1439 cc.
Intravenous injection—55 cc. of 20 per cent acacia solution					
March 12	53	9.30	643	299	20 minutes after injection
March 12	51	9.10	613	298	3 hours later
March 13	55	9.70	675	304	
March 17	51	7.75	608	297	
Dog 17–160. Mongrel. Male. Weight 48.5 lbs.					
March 21	54	7.40	549	251	Blood volume 2166 cc.
Intravenous injection—110 cc. of 20 per cent acacia solution					
March 21	55	5.90	393	208	After injection
March 21	56	6.20	433	212	4 hours later
March 21	47	5.90	390	209	7 hours later
March 22	50	10.20	673	339	

is to be explained by the hemorrhages of the first day due to removal of blood samples.

Table 28 shows the negative results which may follow the introduction of fresh normal dog serum into the circulation of normal dogs. Both dogs show an abnormally high fibrin level and may have had a mild distemper infection. The drop in fibrin values is not quite the expected amount considering the amount of serum injection. The high fibrin values are not again attained on the day after the experiment and

the red blood cell hematocrit still remains low. We have reason to believe that these hematocrit figures of the second day are to be

TABLE 27

Control experiment. Samples removed but no injection

Dog 18–116. Bull. Female. Weight 39 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm*	
March 25	56	6.65	462	201	Blood volume 1867 cc.
March 25	57	7.00	484	210	"After"
March 25	50	6.20	415	206	3.5 hours later
March 26	50	7.60	515	257	

TABLE 28

Intravenous injection of normal serum

Dog 19–114. Fox terrier. Female. Weight 18.8 lbs.

DATE	R. B. C HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
March 11	57	11.7	841	361	Blood volume 892 cc.
Intravenous injection—290 cc. normal dog serum					
March 11	45	7.5	513	283	10 minutes after
March 11	47	7.2	473	250	1.3 hours after
March 11	39	8.7	562	388	4 hours after
March 12	44	11.4	717	399	
Dog 18–92. Fox terrier. Male. Weight 20 lbs.					
March 19	53	13.1	898	422	Blood volume 1041 cc.
Intravenous injection—400 cc. normal dog serum					
March 19	47	8.6	546	289	15 minutes later
March 19	43	7.9	508	290	3 hours later
March 20	41	8.9	582	341	
March 22	41	8.7	558	328	

explained by the removal of many blood samples during the experiment, rather than by a residue of serum remaining for 24 hours increasing the plasma volume and giving a relative decrease in red cells.

Table 29 presents some interesting fluctuations in blood fibrin values. We are not prepared to explain the fall in fibrin values which is noted in both experiments 1.5 hours after the injection of whole blood. We may assume that this induced plethora must be adjusted in some fashion by the body, that all the blood proteins are removed from the circulation to some extent and the fibrin takes its part in this passive reaction. The normal fibrin level is reached 24 hours later.

TABLE 29

Intravenous injection of whole blood

Dog 18–38. Coach. Female. Weight 24 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
February 28	48	10.3	688	355	Blood volume 1197 cc.
Intravenous injection—400 cc. whole blood—donor's blood = 55 per cent r.b.c.					
February 28	51	8.4	564	277	After injection
February 28	50	5.0	340	170	1.5 hours later
March 1	49	9.9	660	336	
March 3	51	9.2	616	304	
Dog 621. Mongrel. Female. Weight 41 lbs.					
March 5	32	6.6	421	285	Blood volume 1614 cc.
Intravenous injection—700 cc. whole blood					
March 5	44	7.0	478	270	After injection
March 5	43	5.1	341	194	1.3 hours later
March 5	39	5.2	343	208	4 hours later
March 6	41	6.9	454	266	
Donor	38	8.5	563	352	350 cc. blood given
Donor	49	7.0	485	246	350 cc. blood given

The blood infusion was done by means of hypodermic needles placed in the veins of both donor and recipient and a well vaselined syringe to transfer and measure the whole blood. There was no reaction following this procedure. In the second experiment the blood fibrin and red cell hematocrit values of the donor's blood are recorded.

Table 30 records two experiments which have been described in part elsewhere (8). It is not necessary to give in detail the experimental

method. This exchange reduces the fibrin level without any complicating shock or reduction of blood serum proteins. As the dog is bled from an artery a similar amount of blood serum and red corpuscles are injected intravenously. A period of 10 to 15 minutes suffices for this procedure which washes out much of the circulating fibrinogen. The regeneration of fibrinogen is prompt and obvious in 3 hours after the exchange. The normal level is usually attained or even exceeded by the end of 24 hours.

TABLE 30

Fibrin depletion—Bled from artery and transferred simultaneously with a mixture of serum plus corpuscles

Dog 19–74. Terrier. Female. Weight 16 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
August 2	58	8.3	600	255	
Exchange = 144 per cent total blood volume					
August 2	49	1.5	75	38	End of exchange
August 2	50	4.4	304	152	3 hours later
August 3	45	8.4	499	281	
August 4	41	11.8	690	411	Infected neck wound
August 5	40	15.0	878	526	Infected neck wound
August 14	25	11.1	636	476	Clean granulation
Dog. Young pup. Weight 15 lbs.					
August 5	42	6.8	435	253	
Exchange = 176 per cent total blood volume					
August 5	45	4.2	305	161	3 hours after exchange
August 6	42	9.9	650	378	
August 7	27	8.4	484	356	Condition good

We note a considerable rise in blood fibrin in the first experiment (table 30) on the third and fourth days following the exchange. This is to be explained by the presence of an infected neck wound. Much more data on infection will be presented in the next paper.

Table 31 cites an experiment which has been described in detail elsewhere (4). A large number of similar experiments has been summarized and we need only state that the fibrin depletion is considerable and that its repair or regeneration is complete within 24 hours. The serum pro-

tein regeneration on the contrary is slow and not completed usually for several days. This experiment is a good example of a large group. The

TABLE 31

Plasma depletion—Bled from artery and infused Locke's solution simultaneously, 800 cc. each

Dog 19–124. Mongrel. Male. Weight 36 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
May 5	50	6.70	457	228	
Bled 800 cc. and infused Locke's solution 800 cc.					
May 5	33	3.40	216	146	3 hours after exchange
May 6	27	7.10	444	323	
May 7	28	9.70	554	400	
May 8	31	10.40	594	403	
May 9	31	11.20	643	405	
May 11	31	11.75	672	464	
May 14	30	10.30	588	412	

TABLE 32

Hemorrhage followed by infusion; bled 725 cc. followed by intravenous injection of Locke's solution, 725 cc.

Dog 19–111. Weight 39.5 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
June 17	46	7.0	402	219	Before bleeding
Bled 725 cc.—Fall in B.P. from 158 to 40 mm. mercury					
June 17	39	5.6	315	193	After bleeding
Intravenous injection of Locke's solution—725 cc.					
June 17	24	4.1	227	172	After injection
June 17	31	5.0	281	194	3 hours later

rise of the fibrin level above normal may be explained in part by slight infection at the operative site.

Table 32 gives the reaction associated with a large hemorrhage and definite shock followed by a saline transfusion. The fibrin level and

TABLE 33

Hemorrhage without infusion

Dog 17–160. Mongrel. Weight 46 lbs.

DATE	R. B. C HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
May 14	55	9.1	644	289	Blood vol. est. 2090 cc.
Bled 530 cc. from jugular					
May 14	52	8.1	558	268	At end of bleeding
May 14	43	6.6	386	220	3 hours after bleeding
May 15	42	8.2	532	307	
Bled 550 cc. from jugular					
May 15	38	6.0	382	236	At end of bleeding
May 15	34	7.4	485	320	3 hours after bleeding
May 16	31	8.8	567	391	
May 19	29	9.2	578	413	
May 20	35	10.8	705	462	
May 21	32	8.0	517	331	

TABLE 34

Sodium nitrite given intravenously

Dog 19–127. Terrier. Male. Weight 29 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
April 30	48	6.6	470	246	
Sodium nitrite—2 cc. of 2 per cent solution in 20 cc. saline					
April 30	44	7.5	517	290	30 minutes later
April 30	41	6.1	499	265	3 hours later
Dog 19–132. Mongrel. Weight 29 lbs.					
May 6	38	6.8	434	269	
Sodium nitrite—2 cc. of 2 per cent solution in 20 cc. saline					
May 6	39	6.8	455	231	30 minutes later
May 6	41	6.6	425	251	4.5 hours later

hematocrit value after the hemorrhage show that fluid has been thrown into the circulation from the tissues. Further dilution of the fibrin content is produced by the saline infusion. The fibrin value shows

TABLE 35

Adrenalin given intravenously

Dog 19–121. Terrier. Male. Weight 30 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
April 22	35	9.0	579	376	Blood volume 1225 cc.
Adrenalin 4 cc. of 1–1000 solution in 25 cc. saline					
April 22	40	8.7	571	342	30 minutes after injection
April 22	38	9.0	584	359	3.5 hours after injection
Dog 19–99. Bull. Male. Weight 36 lbs.					
April 23	48	7.4	452	237	Blood volume 1487 cc.
Adrenalin 4 cc. of 1–1000 solution in 30 cc. saline					
April 23	51	7.7	533	262	30 minutes after injection
April 23	46	7.1	469	252	3 hours after injection
Dog 19–111. Weight 44 lbs.					
April 19	54	9.3	634	293	Blood volume 1103 cc.
Adrenalin 4.5 cc. of 1–1000 solution in 70 cc. saline					
April 19	55	8.4	575	286	After injection
April 19	55	8.0	556	251	3 hours after injection
Dog 17–160. Mongrel. Weight 46 lbs.					
May 19	29	9.2	591	422	
Adrenalin 2 cc. of 1–1000 solution					
May 19	29	9.4	594	421	After injection
May 19	27	9.4	596	433	3 hours after injection

a rise in the next three hours and would have returned to normal within 24 hours.

Table 33 shows the reaction following simple hemorrhage. The dog was bled one-fourth the estimated blood volume on each of two suc-

cessive days. The fibrin value is high and shows a fall during the period of bleeding and during the next 3 hours. This is probably to be explained by the spontaneous dilution of the blood plasma by fluids contributed from the body tissues. After the second bleeding the sharp fibrin regeneration masks the plasma dilution. The normal level is again established on the day after the second bleeding. It may be pointed out that the *whole blood fibrin values actually rise* during this period of bleeding. The effective fibrin values per 100 cc. whole blood are increased This represents the ability of whole blood to form a clot and the toughness of the clot depends upon this figure and not on the plasma fibrin values.

It may be claimed that the changes in the fibrin values noted in many of the above experiments are to be explained by great fluctuations in blood pressure. It has been claimed that increase of blood pressure (exercise, adrenalin) may cause some of the blood plasma fluids to escape and thus explain the observed blood concentration. The experiments given here show that such is not the case. Lowering the blood pressure by sodium nitrite or raising the pressure by adrenalin will not show any greater fluctuations in blood fibrin values than are recorded in control experiments (table 27).

The adrenalin experiments usually show a slight amount of red cell hematocrit increase but the fibrin values are constant. These experiments serve as further control of the experimental procedure.

SUMMARY

Plasma fibrin values may be modified by large intravenous injections of Locke's solution. Subsequently there is a rise above the normal level which may be best explained by the stimulating action of the salts.

Acacia injections will produce similar dilution of the fibrin values depending upon the amounts of solution introduced. This is followed by a slow rise of the fibrin values to normal. There is no over-stimulation of fibrin production.

Control experiments show minimal fluctuations in the fibrin values.

Normal serum given intravenously will cause a reaction much like that observed in the acacia experiments.

Induced plethora by means of whole blood transfusion indicates a removal of blood fibrin during the two hours following the injection. This may be a part of the reaction which adjusts the blood volume to its normal extent under such conditions.

Fibrin may be washed out of the circulation by simultaneous bleeding and intravenous injection of a red cell serum mixture. There is a prompt return to normal fibrin values within 24 hours.

Hemorrhage with or without saline transfusion will cause wide fluctuations in fibrin values but the normal level is usually established or exceeded within 24 hours.

Great changes in blood pressure do not by themselves modify the amount of fibrinogen present in the circulating plasma.

All this evidence speaks for a constant manufacture and utilization of fibrin or fibrinogen in the body. This labile blood plasma protein is entirely different in these respects from the other blood plasma proteins.

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BLOOD FIBRIN STUDIES

IV. Fibrin Values Influenced by Cell Injury, Inflammation, Intoxication, Liver Injury and the Eck Fistula

NOTES CONCERNING THE ORIGIN OF FIBRINOGEN IN THE BODY

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All our experiments in this and other papers emphasize the fact that the blood plasma fibrinogen is a very labile substance which reacts to a multitude of stimuli within the body. It has long been recognized that acute inflammations (pneumonia, peritonitis) were invariably associated with high fibrin or fibrinogen values. It was also recognized that liver injuries were very often associated with low fibrin values, especially when the liver was suffering from extensive acute injuries (chloroform, phosphorus). Many investigators have pointed out normal fluctuations in fibrin values but we believe very many factors were not controlled in these earlier observations. Given a constant diet or fasting and normal health, the dog shows small individual fluctuations (20 to 25 per cent) and in time we may hope to understand even these minor changes in fibrin levels. We have been able to show that certain diet factors may raise or depress the normal fasting fibrinogen level (paper II). The active substances in these foods are not known and more work is needed here.

We have given reasons (paper III) why the blood fibrinogen is being used up rapidly in the daily routine of the body. It is obvious in these experiments how promptly a deficit in fibrin values can be made up in a few hours; this in striking contrast to the other blood plasma proteins (albumins, globulins) which are regenerated only with considerable difficulty (6).

Papers by Whipple (8) and Goodpasture (5) supplement each other and furnish evidence that fibrinogen is produced mainly in the liver but suggest that the intestinal tract may supply a certain amount of reserve

fibrinogen in certain emergencies. The experiments given below furnish additional evidence to explain the complex problem concerning the use and production of fibrinogen in the body. The evidence is very strong that the *liver* is the main and perhaps only source of supply of fibrinogen. It may be well to note the evidence that a certain reserve of fibrinogen is held in the body, perhaps in the intestinal tract epithelium and elsewhere.

The familiar fact that fibrin levels are very high in pneumonia septicemia and peritonitis has been explained as due to the presence of pathogenic organisms in the blood stream and tissues. Our experiments below make it clear that *dead tissue* and a non-specific (aseptic) inflammation are the only factors needed to give this familiar high fibrinogen level in the blood. It is very probable that aseptic or septic inflammation is the cause of extensive deposit or precipitation of fibrin in the disturbed tissues and this brings about a rapid production with an abnormally high fibrinogen level. The fibrin in the lungs of pneumonia is very conspicuous but we have good evidence that much fibrin is deposited within any area of inflammation. This fibrin is rapidly digested by various ferments and is probably continuously formed in the abnormal area, at least during the acute period of inflammation.

EXPERIMENTAL OBSERVATIONS. The various methods and experimental technique have been fully described. Autopsy protocols with a single exception are not furnished as few of the experiments are fatal. Reference will be made in certain experiments to autopsy records given in other papers (7). Unless note is made to the contrary, the dogs are on a mixed diet consisting of cooked meat, bones, bread, rice, vegetables and so forth.

Sterile abscess. A sterile subcutaneous abscess may be produced promptly in a normal dog by the injection of 1 cc. of spirits of turpentine below the skin. A soft, fluctuant abscess, containing sterile pus, forms in the first 24 to 36 hours. It usually measures about 7 to 10 cm. in diameter and will often rupture spontaneously on the third to fifth day. The reaction following this experimental procedure is strikingly uniform in dogs. There is a prompt rise of the blood fibrinogen level as shown in tables 36 and 37. The maximum fibrin values are usually attained on the second or third day and persist until the abscess ruptures or is drained by surgical incision (refer to table 52). If drainage is free, we note a prompt and progressive fall to normal (table 36) but with poor drainage the inflammation does not subside and the fibrin level remains high (table 37).

These experiments and many other similar ones give proof that *tissue injury* and consequent inflammation are alone necessary for this rise in the blood fibrin values. Other experiments indicate that *tissue injury* unaccompanied by the usual inflammatory reaction is sufficient to give this specific physiological reaction—an over-production of fibrin in the body (table 38).

Toxic proteoses. A few experiments with toxic proteoses are given in table 38. The toxic proteose used was obtained from obstructed

TABLE 36

Sterile abscess—turpentine—mixed diet
Dog 19–2. Bull. 25 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
December 30	9.1	45	5.45	327	179	
Turpentine—1 cc. subcutaneously						
December 31	9.6	48	8.00	480	248	Dog active. Abscess forming
January 1	10.0	49	16.40	978	499	Abscess larger and firmer
January 2	9.6	47	14.95	900	474	Abscess 12 × 7 × 4 cm. Fluctuant. Dog slightly toxic
January 3	9.6	42	15.70	926	536	Abscess ruptured. Drainage good
January 4	9.6	42	13.65	805	436	Dog active. Drainage good
January 6	9.2	40	8.80	520	311	
January 8	9.7	42	8.20	483	279	Abscess closed
January 9	9.2	42	8.00	473	279	Dog active
January 11	9.2	45	6.60	394	191	Dog well. Weight 24 lbs.

intestines and its preparation has been described elsewhere (10). The Witte's peptone was obtained in Germany in 1913. These experiments show several interesting points. The first experiment shows a rise in fibrin values and a small drop in red cell hematocrit. The intoxication was moderate and recovery complete in 24 hours. We know that there is an injury of body protein as there is a great increase in urinary nitrogen (9), but there is no familiar inflammatory reaction on the part of any body tissues. This is significant in emphasizing the importance

of *tissue injury* as contrasted with the usual inflammatory reaction. Because the inflammation is conspicuous and much studied its importance as regards the rest of the body has often been overestimated. The *injured tissue* and its disintegration products evidently must be kept

TABLE 37

Sterile abscess—turpentine—mixed diet
Dog 19–21. Pointer, female. 36 lbs.

DATE	WEIGHT	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc Blood	
December 16	Turpentine—1.2 cc. subcutaneously					
	lbs.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
December 17	36.1	56	8.80	540	238	Abscess forming. Dog toxic
December 18	34.0	54	13.70	852	389	Abscess softer
December 19	33.9	45	16.40	964	530	Abscess broken
December 20	34.8	47	14.40	870	465	Poor drainage
December 21	35.3	46	16.00	945	515	Good drainage. Dog better
December 22		50	14.70	879	444	Poor drainage
December 23	38.1	44	11.50	679	378	Abscess opened again. Dog active
December 24	37.6	39	9.00	526	324	Abscess granulating
December 25	35.8	39	7.65	446	273	Dog well
December 26	36.9	40	8.60	507	306	Abscess healing
December 27	36.9	39	8.10	468	287	Wound clean
December 28	37.5	38	7.35	446	276	Dog active
December 30	36.0	40	6.40	364	217	Dog normal
January 3	35.3	45	5.80	344	188	Dog normal
January 6	35.9	48	5.55	331	171	
January 10	35.5	53	5.40	328	156	
January 13	34.5	44	5.70	339	190	
January 20	34.0	55	5.50	349	158	Blood normal

This establishes normal level for this dog.

in mind and wherever possible viewed apart from the familiar inflammation which usually accompanies any extensive tissue injury.

The last two experiments (table 38) show the effects of a lethal dose. The post-mortem findings with the remarkable engorgement of the portal area and especially the intestinal mucosa has been described and pictured (11). We note a loss of fluid to the tissues and a rise in the red cell hematocrit (shock). Under such conditions, we believe there

is a loss of fibrin in two directions: *1*, a passive escape with the other blood plasma elements; 2, an active escape due to the precipitation of fibrin in certain areas sufficiently injured by the proteoses. The high initial level in the last experiment is due to a distemper infection. The reaction, however, is the same following injection of the Witte's peptone

TABLE 38

Toxic proteose intravenously—sublethal and lethal doses

TIME	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
	44	6.60	440	246	
Dog 19–131. Male. 30 lbs.					
10 a.m.	Toxic proteose—intravenous injection—intoxication moderate				
11 a.m.	44	7.90	525	296	Dog toxic, vomited at times
12 m.	42	6.60	445	257	Clinical improvement
5 p.m.	40	7.95	536	322	Dog recovering
Dog 19–93. Male. 17 lbs.					
11 a.m.	41	6.60	462	271	
11 a.m.	Toxic proteose—intravenous injection—lethal intoxication				
12 m.	36	3.80	286	182	Vomiting and diarrhea
12:30 p.m.	47	5.00	336	178	Death. Typical autopsy findings
Dog. Black terrier. 10 lbs.					
	43	9.40	616	352	Distemper
Witte's Peptone, 3 grams—intravenous injection—lethal intoxication					
	45	8.60	560	311	17 minutes after Death in 4 hours

(a mixture of proteoses and peptones). It was necessary to coagulate the plasma by fresh serum.

Roentgen ray injury. These experiments are representative of many similar ones performed in this laboratory to study the systemic intoxication due to the x-ray. These results are to be published elsewhere (7). The x-ray experiments are significant as regards the *intestinal*

tract which has been considered by many as the source of fibrinogen. Others have considered the intestine as contributing to the fabrication of fibrinogen in the liver or as a reserve depot for fibrinogen. Warren and Whipple have many experiments in which large doses of the x-rays given over the abdomen have caused almost complete destruction of the epithelium covering the villi and lining the crypts of the small intes-

TABLE 39

X-ray exposures—fatal and non-fatal
Dog. 19–78. Airedale, female. 30 lbs.

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
March 18	27.2	10.1	66	5.9	384	129	
March 18	X-ray exposure over abdomen—350 milliampere minutes						
March 19	26.7	10.1	60	7.0	436	174	Vomiting
March 20	26.4	10.2	61	10.1	631	247	Vomiting and diarrhea
March 21	25.9	9.9	60	15.0	939	375	Very toxic
March 22	24.9	12.6	66	16.1	994	340	Killed with ether
				Dog 19–101. Airedale			
February 21	30.3	7.1	62	6.8	460	175	
February 21	X-ray exposure over thorax—350 milliampere minutes						
February 22	30.0	6.3	60	8.4	590	234	Normal
February 23	30.2	5.2	55	7.0	499	226	
February 24	29.1	3.9	59	8.2	667	273	Normal
February 25	28.6	11.2	57	7.4	450	185	
February 26	28.1	9.3	55	6.4	400	172	
February 27	28.2	8.0	60	5.1	325	146	
February 28	27.8	10.8	54	6.4	394	157	

tine. These experiments show almost uniform fatal intoxication on the fourth day (table 39) with a *rising curve* of fibrin values. If the intestinal tract were essential for fibrin production, we should expect a prompt fall in blood fibrin similar to that observed uniformly after acute liver injury and extensive necrosis (chloroform).

The second experiment, too, is of considerable interest as the x-rays were given over the *thorax* alone. When exposures of this type are

used, we can demonstrate a definite cell injury in the lymphatic tissues and bone marrow of ribs and vertebrae. There is no acute inflammatory reaction of the usual type and no obvious deposit of fibrin, yet we note a prompt and considerable rise in blood fibrin values. This observation emphasizes again the non-specific type of this stimulus and further the importance of *cell necrosis* rather than any inflammatory reaction. Probably any *cell injury* in the body, with or without any inflammatory reaction, will cause this characteristic reaction with the prompt rise in blood fibrinogen values.

Acute infection. Distemper in dogs is an acute infection which is much like influenza in human beings. Fatal cases usually show pneumonia, but many cases may show normal lungs (dog 18-108, table 40). We note that the fibrin values are usually high—in fact some of our highest figures in dogs are noted in this acute infection (table 40). Also refer to table 9, paper 1, and table 53, below.

TABLE 40

Severe distemper and intoxication

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
Dog. 19–33. Fox terrier, female. 18 lbs.						
September 2	9.4	42	12.2	719	427	Eyes and nose discharging
September 3	9.6	46	13.1	778	422	Weight 16 lbs.
September 4	9.7	43	13.7	809	459	Dog toxic and dull
September 5	9.8	45	16.8	992	547	Death next day
Dog 18–108. Mongrel, female						
		56	25.0	1570	864	Killed with ether Acute distemper without pneumonia

Chloroform injury of the liver. The three tables (nos. 41, 42 and 43) show four experiments—two stimulating doses and two depressing toxic doses, all non-fatal. We followed the general technique established by Davis and Whipple (2) in their chloroform experiments and wish to use the facts established by them in the general argument. The fasting period preceding the chloroform anesthesia makes for uniformity in extent of the liver injury. The first experiment (table 41) with 45

minutes chloroform anesthesia without doubt caused a certain amount of liver necrosis but this injury was not sufficient to cause a fall in fibrin values. This experiment demonstrates again the fact that *tissue injury* or *necrosis* will call out the fibrin reaction and we note a rise during the seven days following the injury. It is probable that hyaline necrosis of liver cells or other body cells is associated with the deposition or utilization of a certain amount of blood fibrin.

TABLE 41

Chloroform anesthesia—stimulating dose
Dog 19–68. "Chow" dog. 13.7 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
January 8	11.0	62	4.9	309	117	3 day fast
January 8	45 minutes chloroform anesthesia					
January 9	10.9	62	5.6	356	135	Fasting
January 10	10.9	62	6.2	390	148	Fasting
January 11	11.0	62	6.0	378	144	Fasting
January 12	11.5	65	8.4	533	188	Fasting
January 13	11.3	59	8.7	478	198	Fasting
January 14	10.9	54	6.7	407	189	Fasting
January 15	10.9	54	7.6	451	210	Crackermeal and rice diet
January 16	11.0	53	5.2	305	143	Crackermeal and rice diet
January 17	10.0	52	6.0	369	176	Mixed diet
January 18	8.4	46	5.5	343	185	Active
January 19	10.4	44	5.2	309	174	
January 20	9.8	45	5.1	308	168	
January 21	10.0	44	5.3	315	175	

Table 42 shows the results of two experiments with one week's interval between the chloroform anesthesias. The first experiment shows that this old dog was more resistant to 60 minutes chloroform than the average dog (compare table 43). It is known that young animals are more susceptible than adults to chloroform injury. The reaction to the first chloroform is a rise in fibrin values (table 42) similar to that observed in table 41. The second chloroform anesthesia of 75 minutes was given after 7 days when it is probable that the greater part of the primary injury had been repaired (3). We note a distinct fall in fibrin values which is in effect for 4 to 5 days. After that the return to nor-

mal is steady and this fibrin curve corresponds in remarkable fashion to the picture of liver cell repair and regeneration which is so clearly understood under these experimental conditions (3). It is to be noted that the curve of fibrin regeneration does not shoot far above normal as observed by Whipple and Hurwitz (12). It is probable that this rise

TABLE 42

Chloroform anesthesia—stimulating and depressing doses
Dog 19–1. Old bull dog. 38 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
December 11	9.3	47	8.2	490	258	3 day fast
December 11	60 minutes chloroform anesthesia					
December 12	9.5	49	8.8	533	270	Fasting
December 13	10.0	47	8.9	553	291	Fasting
December 15	10.8	45	10.1	608	333	Fasting
December 18	9.8	49	11.7	702	359	Fasting
December 18	75 minutes chloroform anesthesia					
December 19	11.2	45	12.0	704	390	Mixed diet
December 20	11.1	48	4.5	267	140	Cholemia
December 21	11.1	42	4.5	211	122	Cholemia
December 22	11.1	44	4.5	264	152	
December 23	10.9	38	4.7	270	167	
December 24	10.7	40	4.8	269	160	Slight cholemia
December 25	9.0	40	5.1	305	183	
December 26	10.8	38	5.7	331	206	Normal
December 27	9.7	38	6.7	406	252	
December 28	10.4	38	7.8	455	281	
December 30	10.1	43	7.9	470	269	
January 2	11.0	44	8.8	518	290	
January 4	10.6	41	7.9	462	271	
January 6	10.4	44	8.1	481	272	

above normal was due to the hemorrhage factor introduced by Whipple and Hurwitz because of the large amounts of blood required for analysis.

The last experiment with chloroform (table 43) gives a prompt fall in fibrin values. The lowest figure in such experiments is usually found on the third day, after which the liver repair is active and reflects its activity in the curve of fibrin regeneration. Cholemia is usually

noted whenever the liver necrosis is of any considerable extent. Observations continued for two months on this dog showed a uniform level of blood fibrin between 280 to 320 mgm. per 100 cc. plasma. Also refer to table 10 in paper 1 for low fibrin values in more severe chloroform poisoning of the same type. We may refer also to the chloroform injury in the series of pups given in table 15, paper II.

Phosphorus and hydrazine injury of the liver. The two tables (nos. 44 and 45) give sample experiments with phosphorus poisoning. The first experiment (table 44) gives a stimulating dose which did not cause any clinical symptoms of intoxication or loss of appetite. There

TABLE 43

Chloroform anesthesia—liver necrosis and low fibrin

Dog 19–66. Fox terrier. 20 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
December 12	10.8	56	5.6	338	151	3 day fast
December 12	60 minutes chloroform anesthesia					
December 13	9.9	59	4.0	250	101	Fasting
December 14	10.0	64	3.1	181	65	Fasting
December 15	10.8	65	2.6	164	58	Cholemia
December 17	10.2	58	3.5	243	105	Cholemia
December 18	10.4	57	4.3	258	112	Fasting
December 19	9.7	61	5.4	345	132	Fasting
December 20	9.8	54	5.1	311	143	Crackermeal and rice diet
December 22	10.0	52	3.4	314	150	Crackermeal and rice diet. Dog active

Observations continued for two months show uniform fibrin level.

was no cholemia. On the second, third and fourth days after the administration of phosphorus subcutaneously, we observe considerable increases in the blood fibrin values. The return to normal is complete in about 1 week which corresponds to the time of complete repair of the injury in the liver. The gradual fall in the hematocrit is not due to the removal of the small blood samples but in large part to the liver disturbance. This accounts for the rise in the fibrin values for the *whole blood.* The second experiment (table 45) shows the reaction following a large dose of phosphorus. This amount (1 mgm. phosphorus per pound body weight) is very close to the lethal dose. We observe a fall in fibrin

TABLE 44

Phosphorus poisoning—stimulating dose
Dog 19–1. Bull, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
March 25	39.0	10.3	45	6.8	400	222	Mixed diet
March 25	Phosphorus in oil—31 mgm.—subcutaneously						
March 26	39.0	10.3	45	8.2	482	261	
March 27	40.3	10.2	45	10.0	589	323	Dog lively
March 28	40.5	9.9	40	9.9	580	346	
March 29	41.2	10.0	44	9.5	559	313	
March 31	38.6	10.5	43	7.6	443	253	
April 1	41.2	10.0	42	7.7	451	262	
April 2	40.0	10.2	37	6.9	400	251	
April 3	39.0	10.9	37	6.8	389	249	Dog normal

TABLE 45

Phosphorus poisoning—depressing or toxic dose
Dog 19–123. Mongrel, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
April 29	30.0	8.5	43	7.2	435	243	Mixed diet
April 29	Phosphorus in oil—30 mgm.—subcutaneously						
April 30	30.2	9.8	43	9.2	542	310	
May 1	30.1	9.7	38	8.7	507	314	Dog quiet
May 2	30.1	9.6	42	4.3	253	147	
May 3	30.0	9.8	38	2.2	128	79	Cholemia—trace
May 5	29.6	10.3	40	3.0	175	105	Cholemia—marked
May 6	29.7	9.5	37	3.3	191	121	
May 7	29.7	9.4	37	4.3	251	158	Dog active
May 8	30.0	9.7	41	5.2	306	179	
May 9	29.0	10.2	39	6.4	371	226	Slight cholemia
May 10	30.0	9.7	40	6.5	381	228	Dog normal

values most conspicuous on the fourth day after the injection. It is interesting to note the initial stimulus on the first 2 days after giving this large dose. The action of phosphorus is very different when contrasted with chloroform. We may say that the type of cell injury is just as different as is the fibrin reaction recorded here. The histological changes parallel in a striking fashion the reaction on the part of the blood fibrin. Cell necrosis is promptly established and very conspicuous in chloroform poisoning. Fatty degeneration is conspicuous and cell necrosis infrequent; further, the injury does not appear promptly after

TABLE 46

Hydrazine sulfate poisoning—toxic dose
Dog 19–124. Mongrel, male. 37 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
April 29	9.8	45	6.0	368	203	Mixed diet
April 29	Hydrazine sulfate—80 mgm. per K.—subcutaneously					
April 30	9.5	58	6.4	417	173	Very toxic. Refuses food
May 1	9.8	63	4.2	271	103	Less toxic. Cholemia
May 2	10.0	60	3.6	225	90	Cholemia marked. Clinically improved
May 3	10.0	58	2.7	167	70	Cholemia marked
May 6	9.2	50	4.4	257	128	Dog normal. Cholemia marked
June 8	9.0	45	4.6	274	150	Cholemia—trace
June 12	10.0	39	5.2	302	184	Weight 33 lbs.
June 18	9.8	47	6.8	421	224	Normal
June 21	9.9	46	8.8	521	279	Weight 37 lbs.

administration of phosphorus. All of this fits in perfectly with the reaction observed in the blood fibrin values. The interdependence of liver injury, liver function and the blood fibrin levels is indeed striking and must have much significance as regards the source of supply of fibrinogen in the body.

Hydrazine sulphate (table 46) is a well-recognized liver poison which in its effects upon the liver resembles phosphorus. We note that the fibrin reaction to a large toxic dose is very similar to that given for phosphorus (table 45). There is the slow steady fall to the lowest level on the fourth day after administration of the drug. What appears to be

a stimulus to fibrin production on the first day is shown by the hematocrit to be the result of blood concentration. The dog was clinically very sick, refused food, vomited and drank much water. In spite of this we note a remarkable rise in the red cell hematocrit which may indicate plasma concentration in spite of liberal fluid intake. This point

TABLE 47

Turpentine abscess—chloroform anesthesia following day—fasting period
Dog 19–1. Dog, old, male bull

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
January 27	9.0	46	8.1	487	265	Weight 35.3 lbs.
January 27	Turpentine—1 cc. subcutaneously					
January 28	9.7	60	10.3	647	261	
January 28	Chloroform anesthesia—75 minutes—3rd day of fasting					
January 29	9.1	53	12.8	788	373	Fasting
January 30	9.0	53	15.9	981	468	Dog toxic. Cholemia Weight 32.6 lbs.
January 31	9.2	52	15.2	918	441	Large sized abscess
February 1	9.6	54	15.6	975	440	Fasting
February 2	9.4	55	13.6	841	376	Abscess full
February 3	9.0	56	11.6	726	323	Abscess ruptured
February 4	9.1	58	11.8	745	311	Good drainage Dog brighter. Weight 29.2 lbs.
February 5	9.3	56	12.0	746	328	Crackermeal and rice diet, daily
February 6	9.6	51	10.1	610	299	Dog active
February 7	10.0	49	8.7	520	265	Weight 30.0 lbs.
February 8	8.8	47	9.3	561	300	Abscess healed Weight 28.0 lbs.
February 9	9.2	47	9.0	551	258	Dog normal
February 10	8.0	46	7.3	438	236	Weight 30.0 lbs.

merits further study. Cholemia is marked during the severe intoxication and present even after clinical symptoms have cleared up.

Abscess plus chloroform injury—combined reaction. Tables 47, 48 and 49 give experiments in which we can study the combined reaction following a depressing chloroform injury and the stimulus of a sterile abscess. In the first experiment (table 47) the turpentine is given on

the second fasting day and the 75 minute chloroform injury on the following day. We are glad to refer to a control experiment on this same dog in table 42. The same chloroform injury was followed by a definite and prolonged fall in fibrin values. It was noted however that this old dog was more resistant to chloroform injury than the average adult dog and we believe this is a constant reaction under uniform experimental conditions (2). There is cholemia which assures us of a considerable liver injury. In spite of this injury we note the usual high rise in blood fibrin values. The rise is well sustained but perhaps not at quite the high level to be expected in an uncomplicated abscess. There is quite a fall in fibrin values before the abscess rupture and this is not often observed in controls without liver injury. From this experiment one might conclude that even the injured liver can respond normally to a stimulus for fibrinogen production (abscess) but we believe this is not literally true. We believe the evidence is that the injury was not sufficiently great to paralyze the liver reaction. It may also be considered that some reserve supply of fibrinogen is drawn upon in this emergency. It seems possible that a great stimulus (abscess) may call out a fibrinogen production which under less stress would not be furnished by the liver.

The second experiment (table 48) shows a simultaneous chloroform injury and abscess stimulus. In this reaction the depressant chloroform injury dominates the situation and we see the usual low level on the second day. In an uncomplicated chloroform poisoning we would expect these low values to persist for several days (table 43), but we see a sharp rise to levels 50 per cent above normal. This would indicate that a great stimulus is able to lift the blood fibrin level considerably above normal even in the face of a considerable liver injury, which is undergoing the usual repair. The liver repair during fasting is known to be considerably delayed and would not be complete for ten days or longer (3). We do not see how this reaction can be explained on the basis of a reserve of fibrinogen in the body because any such reserve would be thrown in *early* in the reaction when the fibrin levels are falling or at the lowest point on the first and second days. This is strong evidence we believe that the injured (or repairing) liver may be associated with low fibrin values but, given a very strong stimulus (abscess) and a non-lethal injury, one may observe a strong reaction in fibrinogen production on the part of the injured liver. In other words, a great stimulus is still able to spur the liver which has suffered a non-lethal injury to great efforts in fibrinogen production. Further, the

evidence is that a *low fibrin value* is not as great a stimulus to fibrinogen production as is the presence of *injured tissue* outside of the liver in the body.

TABLE 48

Turpentine abscess—chloroform anesthesia same day—fasting period
Dog 19–2. Dog, young, female brindle bull

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
January 28		9.8	46	6.8	404	218	
January 28	Chloroform anesthesia—75 minutes. Turpentine—1 cc. subcutaneously. 3rd day fasting						
January 29	24.0	9.0	57	3.4	210	91	Dog quiet. Cholemia marked
January 30	23.4	8.9	61	2.4	154	61	Cholemia +. Abscess fluctuant and small
January 31		9.1	66	3.6	238	81	Cholemia marked Abscess ruptured
February 1	21.7	10.5	58	5.7	346	145	Dog not toxic
February 2		9.8	51	5.7	344	169	Abscess draining well
February 3	20.8	10.0	49	7.8	466	238	Fasting
February 4		9.6	48	8.6	513	267	
February 5	20.2	10.0	49	10.0	595	303	Abscess not draining Fed crackermeal and rice, daily
February 6		9.4	45	10.7	579	317	Cholemia
February 7	20.2	10.0	44	9.4	561	307	Cholemia Drainage poor
February 8	19.7	9.0	41	9.7	576	340	Abscess healing
February 9		10.0	40	8.7	514	303	Diarrhea Abscess healed
February 10	20.4	6.4	42	6.8	428	248	Dog active and well
February 11	20.6	9.2	47	7.2	433	231	Dog active and well
February 12	20.7	9.1	44	6.1	361	202	Dog active and well

We wish to note also the uniform increase in the red cell hematocrit during the acute period of intoxication associated with abscess formation. This same rise in red cell hematocrit is noted in many other similar intoxications and is usually accepted as an indication of loss of circulating fluids. It may be a positive reaction indicative of cell injury and affinity of such injured protoplasm for fluids. We believe it

is a complicated reaction of much importance and hope to report further studies of these phenomena.

In the third experiment of this series, the chloroform was given on the day before the abscess (table 49). Again we note low values on

TABLE 49

Chloroform anesthesia—turpentine abscess following day—fasting period
Dog 19–65. Spitz, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
February 8	22.6	9.8	61	5.4	341	132	
February 8	Chloroform anesthesia—75 minutes—3rd day of fasting						
February 9	21.3	10.1	59	3.5	216	89	Fasting
February 9	Turpentine—1 cc. subcutaneously						
February 10	20.6	10.3	59	2.6	160	65	Dog dull. Abscess forming
February 11	20.1	10.0	59	5.8	360	148	Dog toxic. Cholemia
February 12	20.0	8.8	56	6.5	408	180	Dog toxic. Cholemia marked. Abscess 10 × 10 × 3 cm.
February 13	19.0	9.9	54	6.2	378	174	Condition same
February 14		10.3	53	7.3	441	206	Abscess ruptured
February 15	19.1	10.0	55	9.1	555	250	Abscess draining well. Dog bright. Cholemia
February 16	18.5	10.0	55	7.0	427	192	Fasting
February 17	18.2	10.0	53	7.0	424	199	Fed crackermeal and rice, daily
February 18	18.3	10.0	50	5.5	330	165	Drainage poor
February 19	18.6	7.6	47	4.8	297	156	Abscess smaller
February 21	18.2	8.2	43	5.1	306	176	Cholemia slight
February 22	20.0	8.8	43	5.0	300	171	Dog normal

the second day after the chloroform, due certainly to extensive liver injury but there is a sharp rise in fibrin values immediately after the initial depression. This experiment confirms that in table 48 and supports the conclusions drawn from that experiment. There is opportunity for speculation when one reflects that the liver injured by chloro-

form may be associated with low fibrinogen production but not by reason of necessity, as it is clear that a great stimulus can overcome this lethargy. Does the liver carry this low output during the period of repair and regeneration of new liver cells in order to spare its cell activity or because the same material may contribute to both processes?

TABLE 50

Eck fistula—chloroform anesthesia—stimulating dose
Dog 19–6. Bull terrier, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
February 25	30.0	9.6	59	5.5	342	139	Fasting
February 26	29.0	9.0	54	6.0	373	170	Fasting
February 27	28.5	8.7	60	6.0	388	155	Fasting
February 28	28.0	8.4	61	4.8	314	122	Mixed diet until March 4
March 4	29.0	8.1	60	6.6	433	171	
March 4	Operation—Eck fistula						
March 6		10.0	66	10.1	653	222	Milk and bread diet
March 10	27.4	9.6	58	6.1	381	159	Dog normal
March 11	27.4	10.2	56	4.5	275	122	
March 17	28.1	10.0	54	5.2	316	145	
March 20	26.5	10.1	52	5.3	320	154	
March 21	27.0	10.4	54	4.6	268	128	Dog very active
March 24	25.2	10.1	56	4.2	257	113	Mixed diet
March 25	Chloroform anesthesia—45 minutes						
March 26	26.0		58	5.2	286	120	Dog active
March 27	26.0		59	5.8	365	151	Cholemia
March 28	26.6		55	5.2	318	143	Cholemia—trace
March 29	27.0		58	5.2	320	135	Diarrhea
March 31	26.2	9.8	58	4.9	310	128	Dog active

Tables 50 and 51 give a series of fibrin determinations on a normal dog before and after a successful Eck fistula operation. The autopsy record shows that the operation was successful and the fistula between the portal vein and vena cava was of large size. The portal vein at the hilum of the liver above the fistula was completely occluded by ligature. All portal blood, therefore, was shunted around the liver direct into the right auricle. It is well known that under these conditions the

liver undergoes distinct atrophy and usually if not always presents a central fatty degeneration. There is obvious impairment of function but

TABLE 51

Eck fistula—abscess and chloroform reactions
Dog 19–6. Bull terrier, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
April 1	26.0	10.1	50	5.8	346	175	Bread, milk and bones
April 2		10.1	48	5.4	320	168	
April 3	26.7	10.1	45	6.1	360	198	Normal
April 4		10.0	46	6.1	361	195	
April 4	Turpentine 0.5 cc. subcutaneously						
April 5	25.9	10.2	51	7.7	463	223	Abscess 11 × 8 × 3 cm.
April 6		10.5	50	10.5	625	313	Abscess fluctuant
April 7	24.8	10.2	46	9.7	573	309	
April 8		10.1	46	8.5	495	299	Abscess opened
April 9	25.0	9.6	40	7.2	417	270	
April 10	25.7	9.3	41	7.3	430	256	Abscess draining
April 11	26.2	10.4	44	7.6	450	278	
April 12	27.0	9.4	45	6.4	381	211	
April 14	27.1	9.6	41	6.4	376	223	Abscess draining
April 15	27.0	9.9	44	5.8	343	191	
April 16	26.0	10.1	42	6.1	356	208	
April 17	26.2	9.8	40	4.4	258	154	Abscess healing slowly
April 18	26.2	9.4	41	5.2	307	180	Drainage good
April 19	26.1	10.0	43	5.5	323	184	
April 23	25.0	10.0	47	5.1	303	161	Abscess healed
							Dog active
April 29		10.0	49	6.0	359	183	
April 29	Chloroform anesthesia—75 minutes						
April 30	27.0	9.7	53	5.8	353	168	Dog normal
May 1	24.0	9.7	52	2.2	133	61	Cholemia marked
							Dog very toxic
May 2	23.0	9.7	58	2.4	151	64	
May 3	23.0	9.6	61	2.6	165	64	Dog comatose
							Sacrificed

the condition is not a simple one and the cause of the intoxication which develops sooner or later is not at all understood (13).

The fasting level of this normal dog is constant and there is a rise on the mixed diet (table 50). Following the operation we observe on the second day a marked rise in fibrin values. This is surely to be explained by the operative trauma and wound reaction. The fibrin level after the operation is much below that before operation and this may have significance, perhaps indicating functional impairment. Not sufficient data are at hand to settle this point.

A stimulating dose of chloroform anesthesia gives the expected reaction (table 50). Compare this reaction with that in table 41 where the same type of chloroform injury shows a more striking stimulus and rise in fibrin values. It is to be noted that the dog in table 41 was fasting and in table 50 was not. The extent of the liver injury cannot be estimated and may have been much less than in table 41. The presence of the Eck fistula may further modify the reaction.

Dog 19–6—Eck fistula—autopsy. Autopsy was performed immediately after death and all organs were examined. Only the liver findings will be recorded. The Eck fistula was perfect and the ligature above it at the hilum of the liver completely occluded the portal vein. The liver was smaller than normal and tough. The centers of the liver lobules were yellowish. Microscopical sections show the usual central atrophy in the liver lobules. There are small central hyalin necroses and many wandering cells. Fatty degeneration is marked and involves 30 to 40 per cent of the lobule. The liver cells are "foamy" and severely injured. This necrosis and fatty degeneration involve about one-third of the remaining liver cells. This degeneration is not sufficient in itself to cause death. The remaining liver cells appear normal. There are scattered mitotic figures. Some liver cells show hyalin bile-stained plugs in the bile canaliculi.

Table 51 continues the observations on the same Eck fistula dog of table 50. A sterile subcutaneous abscess is produced and gives about the expected reaction. There is very little difference between the stimulus reaction in the Eck fistula when compared with a normal dog of similar type and weight (table 52). The development of the abscess in each dog calls out the characteristic reaction and prompt rise in fibrin values. There is a prompt fall when the abscess is incised.

Finally the dog (table 51) is given a chloroform anesthesia of 75 minutes—a depressing dose for a normal fasting dog. The Eck fistula dog was not fasting but the injury done the liver was considerable as shown by the autopsy record. The second day after the chloroform, we observe a considerable fall in the fibrin values maintained until fatal intoxication supervenes. The clinical picture did not suggest a fatal late chloroform poisoning and the autopsy confirms this. It is

probable that the usual Eck fistula intoxication was responsible for the fatal intoxication, perhaps precipitated by the liver injury due to the chloroform.

A note should be made concerning the healing of these aseptic abscesses. In the normal dog such abscesses if properly drained will heal in six to nine days. This abscess (table 51) in the Eck fistula dog did not heal with the best of drainage until the 16th day. The abscesses in dogs poisoned by chloroform also were delayed in healing and

TABLE 52

Control for Eck fistula—sterile abscess

Dog 19–66

DATE	WEIGHT	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
April 29	27.0	57	4.5	281	121	Mixed diet
April 29	Turpentine—0.5 cc. subcutaneously					
April 30	27.0	60	5.6	413	167	Abscess 8 × 10 × 3 cm.
May 1		57	11.2	697	298	Dog toxic
May 2	28.8	49	11.0	662	335	Abscess tense
May 5	29.0	51	8.1	478	239	Abscess opened
May 6		44	7.6	451	252	Good drainage. Dog active
May 7		47	7.4	443	233	
May 8	28.6	50	6.0	361	181	Abscess healing
May 9	28.3	44	5.5	336	187	
May 10	28.3	48	5.5	330	170	
May 12	27.0	47	5.0	298	157	Abscess healed
May 14	27.4	50	5.6	336	157	
May 16	27.4	44	5.7	337	187	
May 18		51	6.8	425	209	
May 21	28.4	48	4.6	275	143	

required 13 to 14 days. We have observed that laparotomy wounds in Eck fistula dogs may heal very slowly with sluggish granulation tissue. The same observation concerning laparotomy wounds has been made by Davis and Whipple (4) in their series of dogs poisoned by chloroform. Clark (1) has observed that wounds heal more rapidly when an animal is on a rich protein diet. It is at least possible that other substances besides fibrin which take part in the ordinary wound healing may be influenced directly by the liver injury and food factors.

The last table (no. 53) presents some data on human patients. These figures have some interest when compared with the figures for the normal and abnormal dogs. This series of human cases will be extended in the near future. The normal patients though few in number are very

TABLE 53

Human cases—normal—influenza—blood and liver diseases

TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			DIAGNOSIS	REMARKS
		Average	100 cc. Plasma	100 cc. Blood		
cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*		
8.8	47	5.4	328	175	Normal	
9.6	51	6.0	364	178	Normal	
9.4	51	5.5	333	163	Normal	
10.7	45	5.4	316	175	Normal	
Average.....			335	172		
6.5	85	17.1	1710	263	Influenzal pneumonia	Fatal. Blood urea 96
10.2	43	16.9	987	562	Influenzal pneumonia	Fatal. Blood urea 53
11.0	50	18.6	1099	549	Influenzal pneumonia	Fatal. Blood urea 65
9.8	33	12.6	725	489	Influenzal pneumonia	Not fatal. Blood urea 35
9.9	51	22.6	1366	676	Influenzal pneumonia	Blood urea 51
11.1	59	10.2	689	282	Influenzal pneumonia	Very toxic. Blood urea 53
9.5	55	12.2	753	337	Influenzal pneumonia	Toxic. Blood urea 49
9.4	52	11.8	718	344	Influenzal pneumonia	Very toxic
8.9	34	25.4	1482	992	Influenzal pneumonia	Fatal
11.3	70	4.6	294	88	Polycythemia vera	Cholemia, slight
5.9	42	10.5	680	394	Jaundice	Cholemia +++
9.0	33	11.6	677	461	Suspected cirrhosis	
9.5	33	7.5	431	290	Cirrhosis	Autopsy showed cirrhosis
8.5	58	8.6	546	222	Purpura	Petechial hemorrhages
13.1	18	8.4	458	378	Acute lymphatic leukemia	

uniform in fibrin values. The influenza cases present some remarkably high values with and without blood concentration. The abnormal blood and liver cases are too few in number to permit any generalization. It is suggestive that this case of polycythemia shows a low fibrin level and a somewhat similar finding was recorded in experimental plethora or polycythemia (table 29).

DISCUSSION OF SOURCES OF FIBRINOGEN SUPPLY. A considerable number of factors which stimulate fibrin production or at least cause a rise in fibrin values has been mentioned. These include food factors, hemorrhage, any type of inflammation or tissue injury and even certain general intoxications without obvious cell injury. We may dismiss with a word the theory that fibrin is formed in the bone marrow as it has been shown that essential diseased conditions of the red marrow are not associated with blood fibrinogen abnormalities. Even fatal aplastic anemia shows no fall in fibrin values (8).

It has been thought that the intestinal tract was the essential source of fibrinogen but the extirpation of the entire small intestine does not disturb blood fibrin values and the extensive injury of the epithelium of the small intestine (x-ray experiments, table 39) calls out a very considerable fibrinogen over-production just like tissue injury elsewhere in the body.

Simple hemorrhage causes a distinct over-production of fibrinogen and this is not to be explained by a dilution of the fibrin values alone. We are able to dilute fibrin values in the circulation without causing any reaction if we use acacia solutions or fresh serum. Probably the changes in blood volume are important factors and a *shrinkage* of plasma volume is very apt to bring out an over-supply of fibrinogen. We have good evidence (table 29, paper III and table 53, polycythemia) that *plethora* inhibits the fibrinogen production or at least is associated with abnormally low levels.

Evidence has been noted which indicates a constant daily consumption of fibrin in the normal body (paper III). Also we are familiar with the fibrin deposit which is conspicuous and probably continuous in acute inflammation (pneumonia, peritonitis, abscess and so forth). It is very probable that actual tissue necrosis (hyalin liver or cell necrosis, infarcts and so forth) is associated with considerable deposit of fibrin. Therefore, if the supply of fibrin is cut out and tissue injury is present, we may observe a very sharp drop in fibrin values (acute and severe chloroform liver necrosis).

It is more than possible that there may be certain reserve supplies of fibrinogen in the body and the intestinal tract may be important in this respect (8), (5) but it is unlikely that these reserves can supply any very great amount of fibrinogen over long periods of time.

The only condition which without exception causes or is associated with low fibrin values is *acute extensive liver injury* (chloroform, phosphorus and so forth). The uniformity of this reaction is of great significance and especially the remarkable parallelism which is demonstrable between the extent of the liver injury and the depression in fibrin values. Slight liver injury will cause a rise in blood fibrin values like tissue injury elsewhere in the body but when the injury is extreme and acute we invariably see a prompt fall which usually reaches its lowest level on the second day following the chloroform. This is the period of maximum liver injury and edema with minimum liver repair.

A combination of a toxic or depressant dose of chloroform with a stimulus to fibrinogen production (abscess) gives some very suggestive data. The initial fall (48 hours) in fibrin values due to extensive liver necrosis cannot be obliterated by the abscess stimulus, but the period following this shows the usual rise in fibrin values, persisting until the abscess is drained. This is very different from the usual low level following chloroform poisoning which persists throughout the week or more of liver repair. Evidently then, the repairing or regenerating liver is usually associated with low fibrin values—this may be to conserve cell energy or construction material—but may be spurred to great over-production of fibrinogen by an appropriate stimulus (abscess). This observation makes it very difficult to postulate active fibrinogen production or even extensive reserve supply outside of the liver. It would seem that this emergency reserve would be most obvious at the first of the period when the fibrin values are lowest and if present this reserve is quickly exhausted. We conclude, therefore, that all the evidence points to the liver as the only actively productive source of fibrinogen although there may be certain limited reserve supplies in other body tissues.

SUMMARY

Tissue injury and inflammation exert a powerful stimulus upon fibrinogen production and cause prompt and notable increase in fibrin values. Bacteria are not directly concerned in this reaction, which is identical whether a sterile or septic inflammation is present.

Toxic proteoses cause a fibrinogen reaction which presumably is due to tissue injury although we find no histological evidence of inflammation or tissue injury.

Injury by the Roentgen rays whether over the abdomen (lethal) or over the thorax (non-lethal) gives a prompt rise in blood fibrin values. This thorax exposure with injury of red marrow and lymph cells shows the influence of *cell injury* quite apart from any true inflammation.

Acute infections in dogs (table 40) and humans (table 53) give the familiar stimulus to fibrinogen production and show probably the highest values in blood fibrin.

Small doses of liver poisons (chloroform, phosphorus and hydrazine) are stimulating and cause a *rise* in fibrin values just as tissue injury elsewhere in the body.

Large doses of liver poisons are depressing and if sufficient liver parenchyma is injured invariably show a fall in fibrin values.

A combination of liver injury (depression) with an abscess reaction (stimulus) gives information of value. If liver injury is severe, there will be the initial fall in fibrin values. After the second day of injury, the liver which is regenerating can be stimulated to great production of fibrinogen by appropriate stimuli (abscess). Without this stimulus the liver will maintain only a low fibrin level during its period of repair. The significance of this observation is discussed.

The Eck fistula liver in general reacts like the normal liver in its fibrinogen production. There are minor differences from normal and the Eck fistula intoxication probably has no influence upon fibrin values.

Sterile abscesses heal more slowly in Eck fistula dogs or those poisoned with chloroform than in normal controls. It is possible that fibrinogen or other substances fabricated in the liver play a rôle in normal wound healing.

Plethora in humans (table 53—polycythemia) and in dogs (paper III) experimentally produced is often associated with low fibrin values. This may be a simple reciprocal reaction contrasting with the familiar fibrinogen regeneration resulting from hemorrhage or falling blood volume. Mere dilution of fibrinogen values does not necessarily stimulate fibrinogen production (serum or acacia injections).

Tissue injury is the most powerful single stimulus to over-production of fibrinogen and far exceeds the stimulus of a low blood fibrin level.

There may be certain reserves of fibrinogen or its parent substance held in certain body tissues other than the liver but this reserve is probably not large. We conclude that all available data point to the liver as the only potential source of fibrinogen in the body.

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THE EFFECT OF REPEATED ROTATION ON THE DURATION OF AFTER-NYSTAGMUS IN THE RABBIT

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The use of the after-nystagmus resulting from rotation of the body as a test for the normal state of the labyrinth has in recent years brought this phenomenon into the focus of attention in many quarters. Unfortunately there remains a lack of agreement among observers as to the effects which may result from repeated rotations. Thus on human subjects Fisher and Babcock (1919) report little or no reduction after many days practice in rotation, while Dunlap (1919) gives figures which show a marked shortening of the after-nystagmus. In experiments on animals a like disparity of results appears. Griffith (1920) using the white rat found that after a few weeks of daily rotations the after-nystagmus almost disappeared, but Prince (1920) using cats found no diminution of the after effects as the result of repeated daily rotations.

We have studied the effect of daily rotation on the after-nystagmus in the rabbit. In this paper we shall report the results in ten animals, five of which were rotated with the head free and five with the head confined in a holder.

In these experiments we made use of a specially designed revolving table (see fig. 1) the axis of which is supported on two sets of ball bearings. The resistance due to friction was so small that when set in motion at the speed desired very little change in the rate of rotation occurred during ten turns. In order, however, to be able to control the rate a belt connected a hand wheel, *H*, with a pulley, *P*, on the axis of the table. If the rotation tended to become too slow it could be slightly accelerated by pushing forward on the crank; if the rate was too rapid retardation was secured by a light back pressure on the crank. The belt however was kept so loose that a sudden or abrupt change in the rate could not be produced, the belt would simply slip. In this way it was possible to maintain a smooth and uniform rate of rotation.

The timing of the rate was effected by comparing the ticks of a metronome with the clicking of two wires, W_1 W_2, projecting from the ends of the animal box on the table, as they struck against a fixed, upright wire, W_3. Thus the two wires clicking synchronously with a metronome ticking seconds guaranteed a rate of one turn in two seconds. This was the rate used in the experiments reported in this paper.

It is obvious that the manner of stopping, that is, the rate of the negative acceleration, could have a marked influence on the after effect.

Fig. 1

On this account it was important that the table be always stopped in the same way, and that in doing this the personal equation be wholly eliminated. These results were obtained by the following device. A friction brake, B, was arranged to rub on the outer rim of the table when pressed into position. A cord from the brake was fastened to the axis of the table and was so adjusted that when five turns were wound upon the axis the brake was automatically applied with just the pressure necessary to stop the table at the right place. The table could then be set in rotation only in the opposite direction, the five

turns of cord unwound from the axle, five turns wound on in the opposite direction when the brake was automatically applied as before. With this arrangement the operator could give undivided attention to maintaining a uniform rate, since counting and stopping were automatically performed by the machine itself.

In all cases here reported each rabbit was given ten sets of ten turns to the right and ten turns to the left, two hundred rotations in all, each day, Sundays excepted, until the end of the experiment. In carrying out the experiment one of us usually attended to the rotation of the table and another manipulated the stop watch. At the instant the table stopped the watch was started and was stopped again when the nystagmus ceased. Obviously one had to wait a moment before deciding that no more movements would occur. For this reason the time can not be very accurate. As a check we counted and recorded also the number of eye strokes.

Rotation with head fixed. In these experiments the head was fixed in a Czermak headholder in such position that the rotation was approximately in the plane of the horizontal canal. The results are presented in table 1.

It will be seen that there were marked individual differences at the beginning of the experiment. In all cases there was a definite, though by no means uniform, decrease in the duration of the after-nystagmus and in the number of strokes. In one individual, rabbit I, the after-nystagmus entirely disappeared. All five gave a perfectly normal response to the caloric test at the close of the series. Rabbit I responded as well as any of the others and, for that matter, as well as the rabbits which had not been rotated at all. It is evident then that in rabbits repeated daily rotation causes a reduction in the after-nystagmus.

Rotation with head free. In this part of the experiment the method and the number of daily turns was the same as in the preceding, with the exception that the head holder was not used. In order to keep the animal in position it was placed on a large piece of cheese cloth and when the natural position of the body and legs had been assumed the cheese cloth was wound around a number of times and the animal was then placed in the box on the revolving table, with the head protruding. In this way the legs and body were kept in a natural position, the animal did not struggle and no tying was necessary.

The results of rotation with the head free are in striking contrast with those obtained with the head fixed. In less than two weeks, in

fact in most cases in less than one week, the after-nystagmus had practically disappeared, as is shown by table 2.

It should be mentioned that when the after-nystagmus following rotation at the rate used in these experiments had wholly ceased to appear, a vigorous after-nystagmus could be obtained by using a more rapid rate, say 10 turns in 10 seconds.

TABLE 1

Average duration in seconds, S, and average number of eye strokes, N, of the after nystagmus for each day of rotation of each of the five rabbits, 1, 2, 3, 4 and 5 respectively, rotated with head fixed

DAY	NO. 1		NO. 2		NO. 3		NO. 4		NO. 5	
	S	N	S	N	S	N	S	N	S	N
1	1.8	1.8	3.2	6.3	9.2	14.1	7.6	14.7	16.8	20.1
2	0.5	0.5	4.0	6.8	5.7	12.4	4.8	10.1	10.9	23.8
3	2.1	0.7	4.7	11.0	6.1	15.3	4.8	9.5	8.2	17.7
4	0.3	0.2	5.9	13.6	6.2	13.0	4.9	9.9	9.9	20.0
5	0.2	0.2	5.4	13.4	7.0	14.1	6.1	12.3	8.5	14.2
6	0.4	0.3	5.6	12.2	6.3	13.0	6.5	13.5	7.8	17.2
7	0.1	0.3	5.2	12.1	5.8	12.6	5.1	10.0	8.1	17.3
8	0.1	0.1	5.0	10.1	5.9	12.2	5.5	11.5	6.7	14.9
9	0.1	0.2	4.8	10.3	5.6	11.8	4.8	11.5	8.1	16.4
10	0.1	0.1	5.0	9.9	5.6	11.7	4.7	10.1	7.5	16.1
11	0.1	0.1	4.1	7.7	5.1	11.3	3.7	8.5	8.1	16.0
12	0.1	0.1	3.4	7.0	4.4	8.3	3.9	8.5	6.5	14.1
13	0.1	0.1	3.8	6.5	4.2	8.6	4.1	8.6	6.5	12.9
14	0	0	3.5	7.1	4.5	9.8	3.5	7.7	6.8	13.8
15	0.1	0.1	3.1	6.3	5.5	12.7	3.5	6.6	5.1	14.1
16	0	0	2.9	4.9	4.4	8.9	2.9	4.9	5.0	10.2
17	0.2	0.2	4.1	7.4	4.5	9.3	1.9	3.6	3.5	8.1
18	0.2	0.2	2.9	5.7	4.2	8.8	1.0	1.2	4.0	8.5
19	0	0	2.8	3.8	4.1	6.2	2.5	3.6	2.7	5.5
20	0.1	0.1	2.5	5.0	3.4	6.2	0.3	0.3	2.4	4.7
21	0.1	0.1	2.2	3.6	4.2	7.8	0.1	0.1	2.4	4.7
22	0	0	1.5	4.1	4.9	9.4	1.3	1.9	2.5	4.3

Tested with the cold water douche, rabbit 8 gave very little response but in the others the responses were normal. It is questionable whether even no. 8 was pathological so far as the labyrinth is concerned, for obstructions in the auditory canal may have prevented entrance of the water to the level of the drum membrane. Unfortunately we did not investigate this in this particular specimen, but it has been found by one of us that such obstructions often occur in otherwise apparently

normal animals, and hence the absence of the caloric reaction can not be taken without reserve as evidence that the labyrinth is not functioning.

It was to be expected that rotation with the head free would produce a less marked nystagmus for the following reasons: As soon as rotation begins a compensatory position of the head is assumed. Thus on rotation to the right the rabbit's head goes strongly to the left.

This position of the head now exerts an effect reflexly on the eye muscles. Lyon (1900) reported an observation of Garrey's that when the head of a dogfish is bent to one side the eyes assume a complementary position. Thus when a dogfish comes to rest against the wall of an aquarium so that the head is turned to the right the eyes are turned a corresponding

TABLE 2

Average duration in seconds, S, and average number of eye strokes, N, of the after-nystagmus for each day of rotation of the five rabbits, 6, 7, 8, 9 and 10, respectively rotated with head free

DAY	NO. 6		NO. 7		NO. 8		NO. 9		NO. 10	
	S	N	S	N	S	N	S	N	S	N
1	4.6	7.7	7.1	7.2	6.9	9.9	4.4	2.9	4.9	3.5
2	3.7	3.4	0.8	0.7	4.6	7.1	3.0	2.2	3.0	3.0
3	1.0	1.0	0.3	0.4	2.2	1.8	0.2	0.1	0.4	0.2
4	0.7	0.7	0.3	0.2	1.1	1.0	0.1	0.1	0	0
5	0.3	0.3	0	0	1.8	1.3	0	0	0	0
6	0	0	0	0	1.1	0.7	0	0	0	0
7					1.2	0.9				
8					1.1	0.7				

amount to the left. Bárány (1906) made a similar observation on the rabbit. If the head of a rabbit is firmly fixed, so that labyrinthine reflexes are excluded, turning the body to the right causes deviation of the eyes to the left. If the turning is continued till an angle of 90° has been reached, the eyes reach a maximum left position and come back with a quick jerk; this is repeated about three times and constitutes a characteristic nystagmus with the slow component to the left, that is, to the direction opposite to that of the bending. Finally de Kleyn (1918) has shown that this, in the rabbit, is a reflex from the joints or muscles of the neck, for it disappears after section of the dorsal roots of the cervical nerves.

When a rabbit is rotated to the right, if the head is free to move it at once turns to the left. The tendency of the rotation to the right

is through the labyrinth to produce a nystagmus with the slow component to the left; but the position of the head to the left tends, as Bárány's experiment shows, to produce a nystagmus with the slow component to the right. The effect then of the head position is in part to neutralize the reflex from the internal ear, and the actual result is therefore the algebraic sum of two quantities of opposite sign.

In order to test the correctness of the above deductions we made some experiments in which rabbits were rotated with head fixed at an angle with the body of about seventy degrees. The averages of two sets of rotations are shown in table 3.

It is seen from this table that in the position assumed by the head during rotation, a position which is always contrary to the direction

TABLE 3

Average duration in seconds, S, and average number of eye strokes, N, of the after nystagmus for two rabbits rotated with head fixed at an angle of 70 degrees. Each number is the average of five tests of 10 turns in 20 seconds

HEAD TO RIGHT		HEAD TO LEFT	
S	N	S	N
Rotation to right			
6 6	12.0	4.4	9.4
7 0	14.0	4.3	8.4
Rotation to left			
3 9	7.0	6.5	11.1
4 0	7.2	6.4	11.0

of rotation, the after-nystagmus is less than when the head is turned the opposite way. This experiment shows why the ocular effect is less when the head is free than when fixed, but we must admit that it does not in itself explain the rapid decrease in the response to rotation.

CONCLUSION

Our experiments show that daily rotation of the rabbit reduces, in some cases very markedly reduces, the after-nystagmus. We have attempted by the caloric test to discover the functional state of the labyrinth at the close of the series. We have no reason to believe that the animals have been injured by the amount and rate of rotation to which they have been subjected. It is true that Wittmaack (1909),

has reported and de Kleijn and Magnus (1920) have confirmed the statement, that rotation (centrifugalization) at a high rate of speed can produce destructive changes in the labyrinth. But the latter observers have used even this high speed to differentiate between the canals and the otoliths, since only the otoliths are injured by this means. Now in our experiments we are dealing with reactions to rotation in the plane of the horizontal canals, in which we believe the ampullae of the horizontal canals are mainly and the otoliths possibly not at all concerned. If it were a matter of injury and not merely habituation a peculiar question would yet remain unanswered, namely, Why is rotation more injurious to the animal with head free than with head fixed? We believe the results warrant the assumption that no injury has been done but that through habituation the organism has been rendered less responsive.

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THE CONTOUR OF THE VENTRICULAR VOLUME CURVES UNDER DIFFERENT CONDITIONS[1]

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In several recent communications from this laboratory (1), (2), (3) evidence was presented to show that, under varying conditions, the duration of the ejection phase is modified by factors other than the duration of preceding diastole. The conclusion naturally followed that the volume curves of the ventricles cannot be superimposable under such conditions of the heart beat. These deductions should, however, be capable of direct demonstration when ventricular volume curves are taken during similar changes in the circulation; and, in addition, the precise character of the alterations in filling and expulsion accompanying such phasic changes should be determinable, provided, of course, that it is possible to record changes in systolic ejection and diastolic filling with a sufficient degree of accuracy.

Before proceeding with a narration of the results obtained in such an investigation, it is important to delineate, as they appear to us, the principles and practice concerned in volumetric studies of the ventricles. It is desirable also to analyze the character of representative volume curves and to point out the features which readily lend themselves to misinterpretation.

THE PRINCIPLES AND PRACTICE OF GRAPHIC REGISTRATION OF VENTRICULAR VOLUME CHANGES. A discussion of this topic may be resolved into two portions: *a*, the ability of an apparatus to faithfully reproduce such volume changes as occur in a cardiometer; and *b*, the correspondence of plethysmographic volume changes and actual volume changes of the ventricular cavities.

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Accuracy in recording rapid plethysmographic volume changes such as occur in cardiometers. The principles according to which efficient volume recorders should be constructed are well understood (cf. Brodie (4), Henderson (5), Straub (6)). Fundamental emphasis is laid by investigators on two important requisites: *1*, the necessity of avoiding more than minimal variation of pressure within the system, and *2*, the maintenance of an adequate inherent vibration frequency. Two types of apparatus, as Brodie points out, have been utilized: *a*, *true volume recorders* in which the internal pressure variations are very slight, and *b*, tambours in which volume changes are translated into pressure changes and the latter recorded. Most experimenters seeking to avoid what is usually regarded as a deleterious effect of negative pressure variations about the ventricles, have endeavored to use true volume recorders. With the possible exception of the soap bubble device suggested by Straub (6) and later (11) found impractical by him, it is not possible to construct recorders which qualify as regards inherent frequency.

We are of the opinion, however, that the development of moderate negative pressure variations about the ventricles during the heart cycle is not detrimental to their normal action; that, on the contrary, it is quite normal and favorable. The heart is naturally enclosed within a plethysmographic cavity—the chest, and every volume change is accompanied by pressure variations about the ventricle. Since actual measurement (7) has shown that, in addition to the negative pressures exerted on the ventricles during inspiration and expiration, each ventricular systole itself causes an intrathoracic pressure change ranging from 9 to 20 mm. of water, natural conditions are but reduplicated when similar changes occur about the ventricles in a cardiometer. In other words, we distinctly favor the use of an apparatus which translates volume changes into moderate tension changes. If such an apparatus is used we are in a position to improve its vibration frequency. Fortunately the requirements as to frequency are not great. In the dog, at least, cardiac filling and ejection are comparatively slow processes; there are no rapid vibrations such as occur in pressure records. Since the systolic ejection is probably the most rapid phenomenon recorded and its duration is rarely less than 0.1 second, an instrument with an inherent frequency of 15 per second is probably quite adequate.

In applying these principles we returned, after many trials of various forms of apparatus, to the segment volume recorder presented

in picture form by one of us in 1915 (7). The volume changes within the cardiometer were communicated by tubing 12 mm. internal diameter and 60 cm. long to a tambour 9 cm. in diameter constructed on the principle of a segment capsule. When this segment recorder was covered by thin rubber dam, smoothly fastened over the surface without stretching, it was found that 30 cc. displacement caused a pressure variation of 31 mm. of water. By attaching to the trapezoidal plate pivoting on its chord side a straw lever 12 cm. in length and magnifying 3 times, a graphic record could be inscribed on a smoked surface. In order to inscribe the systolic ejection as a downstroke, the apparatus was inverted. Except for the distortion incurred by arcs of levers,

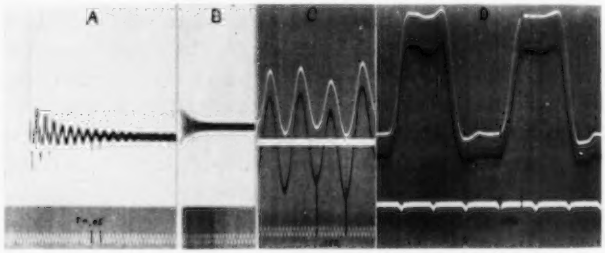

Fig. 1. Four segments of records illustrating dynamic tests made on apparatus described in text (⅓ actual size).

A: Test of vibration frequency; N = 20. Smaller vibrations of much greater frequency superimposed on waves are partial vibrations of optical segment capsule.

B: Vibration frequency of segment capsule; N = 100.

C: Correspondence between piston movements of a syringe (white and optical records (black) when variations of volume exceeding 375 per minute were produced.

D: Records showing correspondence of slower and more extensive volume changes. Deflection time about 0.10 second. Note absence of overshooting and that delay in conduction is negligible.

such records are very reliable as to details. In order to relate the volume changes to pressure curves which can not be inscribed with accuracy on smoked surfaces, it was necessary for our purpose to record optical volume curves as well. To accomplish this, the slight pressure variations within the large capsule were communicated to a Frank segment capsule, as shown in the illustration referred to above. This apparatus fulfilled all the demands made upon it and was suitable either for graphic records on smoked paper or optical tracings or what

proved desirable for our experimental work—both together. Throughout our experiments it was thus possible to record continuous volume curves on a slow drum, to note changes in systolic or diastolic volumes, changes in adjustment, etc., while optical tracings were taken at intervals electrically marked on this drum.

Dynamic tests showed that the closed system in which a tambour 9 cm. in diameter was used, had an inherent frequency of approximately 20 per second (see fig. 1, *A*). This instrument proved suitable for medium sized dogs which were employed by us exclusively. Tests in which the rapidity of response to large volume changes was determined showed that the deflection time is much less than 0.1 second and that no overshooting or lag occurs (cf. fig. 1, *D*). Upon testing its ability to react to rapid volume changes, a test suggested by Henderson (5), it was found that it followed the shadows of a lever attached to the handle of the moving piston even when this was operated at the rate of 380 per minute (cf. fig. 1, *C*). We are therefore convinced that this arrangement faithfully reproduces such volume variations as may occur in the cardiometer.

The correspondence of cardiometric volume changes to those of the cardiac chambers. In order to represent the details of ventricular filling and expulsion even approximately, it is important that the volume curves should not be distorted by other factors or that these should at least be discountable in the analyzed records. We beg to waive any extensive review of the many different opinions already expressed on this subject except as they bear directly on the points under discussion. As Henderson pointed out, changes in extra-ventricular volumes are dependent to some degree on the varying blood volume contained in the coronary vessels. It is generally agreed, however, that this factor is of minor significance during the acts of ejection and filling. In addition all workers have recognized that certain extraneous volume changes are apt to be set up as the heart changes its position during the cycle. Henderson (5) has directed attention to the possibility that auricular systole tends to push the heart farther into the cardiometer. Patterson, Piper and Starling (8) believe that it exerts a traction and thus halts the increase in volume just previous to auricular systole. Henderson believes, however, that such auricular changes can be prevented by proper placement. We agree that this is quite possible. Straub (6) and Gesell (9) believe that the volume changes may be inexact at the onset of systole due to movements of the a-v valves. They point out two effects that may operate,—a bulging of the a-v valves

during the isometric phase, which tends to decrease the oncometer volume, and a downward pull of the valves by the papillary muscles which operates to increase it. Records of intra-auricular pressure (figs. 6 and 13) clearly show that the predominant effect is a sharp reduction of pressure indicating that the chief movement of the a-v diaphragm is toward the ventricle.

We have from the onset been particularly impressed with the possibility that a position change of the heart at the beginning and end of ejection may affect the reliability of volume curves. If the cardiac base of a heart freed from its pericardium is observed carefully, it will be seen that, during each systolic kick, the base of the conical ventricles is pushed farther into the cardiometer thereby increasing its volume and that, at the beginning of diastole, it recedes again to its former position, causing a reverse effect.

After two months of daily experimentation in addition to some previous experience, we have concluded that, in many animals, this systolic thrust and diastolic withdrawal cannot be satisfactorily eliminated by any technical procedures and is probably a factor to be reckoned with in the interpretation of volume curves. The magnitude of the accidental volume change varies in different animals, being determined, among other things, by the vigor of the heart and the pressure conditions in the large arteries. If a heart is beating quietly or if arterial pressures are relatively low, this thrust is not obvious to the eye but still recordable by special magnifying devices. If the heart is vigorous, no recorded proof of its existence is required.

Another accidental volume change occasionally complicates this work. The theory of cardiometry presupposes that it is possible to fit a rubber diaphragm, air-tight and without compression, in the plane of all valvular orifices. We have found it exceedingly difficult to do this in some animals because the pulmonary valves lie on a lower level than those closing the a-v ostia. In such cases a portion of the pulmonary artery readily comes to lie within the cardiometer and, in such event, the early systolic volume changes represent the difference between decreased volume of the ventricle and increased volume of the included artery.

Furthermore, the pulmonary conus below the valves sometimes swells during the early phase of diastole apparently because the semilunar valves bulge into the ventricle after closure. These phenomena may cause an increase in plethysmographic volume even though diastole has begun.

We are therefore of the opinion that, while it is quite possible to record directly and accurately such volume changes as occur within the plethysmograph, these changes may not represent a true picture of intraventricular volume changes, owing largely to the inability to keep a vigorously kicking heart entered into the cardiometer to a constant depth. As long as the movement of the cardiac base into and out of the cardiometer can not be controlled further improvement in recording appliance will accomplish nothing, nor can tachygrams such as have recently been recorded and integrated by Straub (10) be considered more accurate than good volume records. The only question at issue and meriting further analysis is whether the accidental distortions thus

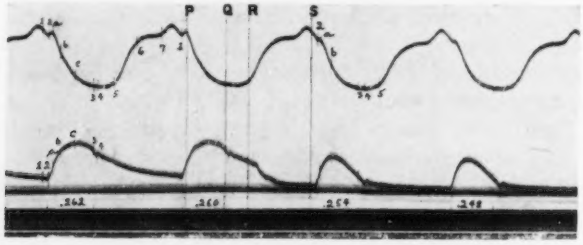

Fig. 2. Volume and aortic pressure curves during and immediately following aortic decompression; used to illustrate ideal curve in the latter condition and production of an early systolic peak, *2-a*, due to kick of heart when arterial resistance becomes high. Small numerals and intersecting lines on curves of this and subsequent illustrations demarcate the following phases: *1–3*, entire systole; *3–1*, entire diastole; *1–2*, isometric phase (approximately); *2–3*, systolic ejection (subdivided into shorter phases, *c*, *b*, *c*); *3–4*, proto-diastolic phase; *4–5*, isometric relaxation phase; *5–6*, rapid inflow phase; *6–7*, diastasis phase; *7–1*, auricular systole phase. Note abbreviation of systolic ejection on decompression. Lines *P Q* and *R S* indicate portion of records considered reliable in volume curves. (⅓ actual size).

produced can be detected on the volume curves and discounted with sufficient accuracy to permit of their use in studies of contour changes.

The nature of optically recorded volume curves.[2] By recording ventricular volume curves synchronously with pressure changes in the cardiac chambers and large vessels and at times also with records of the movements of that portion of the cardiac base to which the rubber diaphragm

[2] Inasmuch as the illustrations have necessarily been considerably reduced in size it is suggested that they be examined with a hand lens, in order to detect the finer details brought out in the text.

was applied,[3] we have been able to interpret a large number of volume curves of varying contour. The fact that this series includes specimens simulating published volume curves of practically all investigators and that our interpretation does not always agree with theirs would in itself be sufficient grounds for submitting the more prevalently published types to further interpretation.

Our aim, however, is not to present a critical review of various interpretations but to establish for ourselves a method of evaluating the contour changes we have obtained under different conditions. The first is incidental, the latter necessary.

In analyzing the different "contour types" of volume curves contained in our collection and also in the published records of other investigators, it is important to recognize differences that are *apparent* and not real. The impression of contour given by any recorded curve is of course dependent on the relative ordinate and abscissal values. Usually optical records are taken on a more rapidly moving paper than drum tracings, thereby increasing the abscissal distances without increasing the ordinate values correspondingly. This gives the smaller records the appearance of having a rapid downstroke with a prolonged flat bottom while the records of larger amplitude appear distinctly rounded (fig. 3, *A*). Neither may at a glance resemble drum tracings. Careful study shows, however, that all records present the same changes in gradient. It is especially important to recognize these differences in optical tracings for, in order to record large volume changes under abnormal condition, it is often necessary to start with small normal curves in order to keep the larger excursions on the paper. Occasionally these records with smaller amplitude give the entire curves quite a questionable appearance. This is the case in some of Straub's (11) published tracings (fig. 7), duplicates of which we have in our possession. In these a sharp downstroke appears to change suddenly to a very gradual slope. If such curves are replotted with larger ordinates and the same abscissas, the familiar contours reappear.

There are, however, real differences in contours which are due in part to variations in circulatory conditions and in part to accidental causes which it is important to analyze.

[3] Proper attention was given to parallax in these registrations. Our camera, provided with a deep narrow hood in front of the lens, failed to record any record more than 0.75 mm. out of alignment. As additional precautions, the relative positions of writing beams were further photographed through amber glass on a stationary drum. It would be needless to add this detail were it not for the fact that some of our interpretations hinge especially on this point.

The intrinsic variations and accidental distortions of the systolic stroke. In order to check the accuracy of volume changes during systolic ejection, the aortic pressure curves near the semilunar valves must be simultaneously recorded. This is important, not only because it is thus possible to determine the beginning and end of ejection precisely, but also because the contour of the volume curve can really be predicted from changes in the gradients of the pressure curves. If the gradient of the pressure curve changes suddenly during ejection this can only mean that the rate of ejection has altered. If the volume and pressure curves thus related, agree, direct proof of the accuracy of the volume curve is given; if they do not show such a correspondence their accuracy must be questioned.

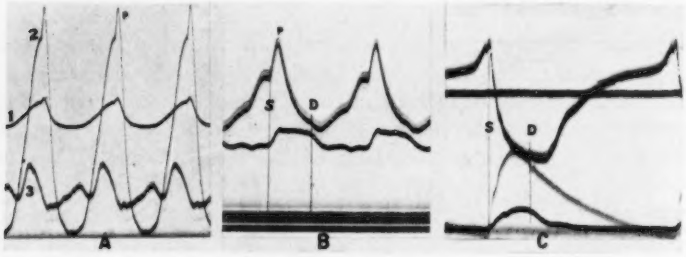

Fig. 3. Curves showing some causes of deformation in volume curves. (⅓ actual size).

A: Apparent differences in contour due to records of different amplitude recorded on same rate of drum; *1*, curve with heavy rubber membrane; *2*, simultaneous curve with delicate membrane; *3*, right intra-auricular pressure curves showing that auricular systole comes just at end of inflow phase thereby slightly increasing inflow rate. Observe accentuation of ejection peak, *P*, in curves of larger amplitude.

B: Serious distortion of curve due to movement of heart into and out of cardiometer. Upper curve, volume record of ventricle with large ejection peak, *P*, due to movement of heart into cardiometer, and with continued decrease during early diastolic phase due to movement of ventricles out of cardiometer. *S*, beginning of systole; *D*, end of systole. Lower curve, record of cardiometer diaphragm movement. Upstroke, signifies movement of diaphragm inward; downstroke, outward movement. Note the minimal effect of auricular systole on diaphragm.

C: Minimal distortions of record when in and out movement of heart occurs more gradually. Upper curve, volume curve of ventricle with very slight peak and very slight movement at end of systole. Lower curve, movement of cardiac base. *S*, beginning; *D*, end of ventricular systole. Ventricular volume curve shows gradient changes corresponding to those in aortic pressure curve.

It is obvious that, with the beginning of the rise of aortic pressure, the volumes of the ventricles must decrease. Precise time relations show, however, that this is rarely the case (e.g., fig. 3, *C*). More commonly the ventricular volume curves either remain unchanged (fig. 2, 3rd beat) or increase slightly during the early moments of ejection (fig. 2, 2nd beat). In the latter case, the curves develop a small peak which is also indicated in many published records of other investigators. This slight increase in volume has usually been interpreted as taking place during the isometric period, being frequently held to indicate a pull of the papillary muscles on the a-v valves (cf. e.g., Straub (10)). We have measured a large number of records carefully and find, contrary to other investigations and our own previous conception, that this notch only rarely precedes but usually comes at onset of ejection. Our experiments indicate that this is an accidental phenomenon due to a slight systolic thrust of the heart into the cardiometer. In the first place, we noted that this peak is brought out or accentuated when the activity of the heart or the arterial resistance increases (cf. figs. 2, 9, 10). Furthermore synchronous records of the basal portion of the heart or cardiometer diaphragm showed that this variation is always accompanied by a movement of the heart into the cardiometer. That, under certain conditions, this movement may begin during the isometric phase is conceivable, but under the conditions of our experiments this movement coincided rather with the onset of ejection as shown in figure 3, *B* and *C*.

It is quite obvious that the volume changes recorded during the isometric phase or during the very beginning of ejection cannot accurately represent actual volume changes in the ventricles. If the peak is small, however, it interferes little with the interpretation of the rest of the record; if it becomes broad and large as in figure 2, *B*, care must be exercised not to interpret it (as has occasionally been done) as an effect of auricular systole.

A comparison of contours shown by synchronous volume and pressure curves gives direct assurance, however, that the details of the remainder of systolic ejection (e.g., *P-Q*, fig. 2) are accurate. Curves, such as shown in figures 2, 3 *c*, 5, 9 and 10 show that the gradient of the ejection curve corresponds exactly to changes in gradient of the pressure curves. In all cases there is an abrupt decrease in ejection rate at the time that the aortic pressure begins to decline, thus separating systolic ejection into phases of maximum and reduced ejection. Thus, in figure 2, *2-c* represents the phase of maximum ejection and *c-3* that

of reduced ejection. Further consideration shows however, that the velocity of ejection is uniform during the phase of maximum ejection only when arterial resistance is low and the aortic pressure curve mounts smoothly to a summit (fig. 2, 3rd and 4th beats). When resistance is at or above the normal level, we find, contrary to Henderson and in accord with Straub, that the velocity of ejection may change a number of times, depending on the resistance developed in the arteries from moment to moment. Contrary to Straub, we believe it impossible to select any type of variation as normal, there being as many variations of ejection during this phase as there are types of pressure curves. Thus, in the first beats of figure 2, the velocity of ejection is very rapid during the primary elevation of aortic pressure, *a-b*, and is somewhat reduced as the pressure curve gradually reaches a summit, *b-c*. In records such as figure 5 there may be several changes in velocity during the maximum ejection phase.

Contour changes during the protodiastolic and isometric relaxation phases. The term, *protodiastolic* phase, has recently been applied by one of us (3) to the interval occupied by the incisura or closure of the semilunar valves; while the relaxation process occurring when all valves are closed, was referred to as the *isometric relaxation phase.* These phases are shown on all records by lines *3-4* and *4-5* respectively.

Since blood neither leaves nor enters the ventricles during these stages, the ventricular volumes do not actually change. This is the case in many records (e.g., fig. 2). In other instances the only change is a slight increase (e.g., fig. 5, *C*, *D*) possibly due, as Henderson suggested, to the filling of the coronary vessels. Sometimes, as in Henderson's records, this increase is much greater and the suspicion then exists that the rebound of blood in the pulmonary conus may contribute somewhat to the distortion. Of greater importance because it readily leads to misinterpretation of volume curves is a continued decrease in volume during these phases (figs. 3 *B*, *9*). Without the time relations such as given by the aortic pulse or heart sounds, the end of systole is readily interpreted as occurring at *5* rather than at *3*. A similar notch evidently wrongly interpreted as part of the systolic ejection process appears in the work of Patterson, Piper and Starling (8). The cause of this continued decrease during early diastole is clearly due to a return of the cardiac base to its diastolic position (fig. 3, *B*). Indeed it is not improbable that a horizontal curve during these phases (fig. 2) is not due to the fact that ventricular volumes do not change but owes it existence rather to a nice counterbalancing of two factors; the move-

ments of the heart out of the cardiometer decreasing the cardiometric volumes and the filling of the coronaries and pulmonary conus increasing the volume to an equal degree. Indeed the slope of this portion of the curve may change within a few consecutive beats when circulatory conditions are altered; e.g., figure 9, *A*, where an upward slope from *3-5* changes to a downward slope after aortic compression. Since no cardiac filling takes place during this interval, however, such accidental distortions are of no practical significance, provided time relations are accurately established.

The volume changes during diastolic filling. The accuracy of diastolic filling curves may be controlled by simultaneous records of left and right auricular pressures. Such controls, as well as records of the oncometer membrane (fig. 3, *B*) show that this portion of the curve is fortunately not distorted by position changes of the heart. The filling changes, as first pointed out by Henderson (5) and reëmphasized by one of us (3) recently, depend on the duration of diastole. If, as in figures 6 and 13, *A*, the heart is rapid, filling has scarcely begun before the next auricular systole supervenes and in such cases ventricular filling may occur entirely during auricular systole, which, it should be recalled, lasts both during the rise and fall of the auricular pressure wave. Consistently the rise of right auricular pressure precedes, by a short interval, that of the left (fig. 6). It is obviously impossible to evaluate the extent to which auricular systole aids ventricular filling in such cases. Such an evaluation may be clearly made however in such records as are shown in figure 13, *B*, *C* and *D*. Here diastole is very much prolonged and two auricular systoles come during one of these diastoles (2:1 block). In figure 13, *B*, e.g., the early diastolic inflow phase coincides with the fall of auricular pressures, *5*. This inflow stops short, however, as indicated at *6*, where diastasis begins. Precisely at this time the auricular pressure begins to rise slightly and the volume curve becomes more horizontal. This marks the beginning of diastasis. Another auricular systole then supervenes at *x*, which adds a fair contribution to the ventricular filling. This is followed by a continued diastasis until the next auricular systole occurs. Such curves clearly bear out Henderson's observations as to the existence of a diastasis phase under normal conditions. As to the volume contributed by auricular systole, this appears to be inconstant, estimates on our curves ranging from 18 to 60 per cent with an average of about 35 per cent. It is apparently dependent, *a*, on the time that auricular systole

comes in diastole; *b*, on the completeness with which the ventricle has already been filled at the time of auricular systole, and *c*, on the vigor of auricular systole. The changing volumes contributed under different conditions will be discussed later. It may be pointed out at once, however, that, other things being equal, an auricular systole coming during or soon after the early diastolic inflow phase contributes a considerable amount of blood. This agrees also with records of Straub (10), (11), and de Heer (12). Coming at the end of a long diastasis, it is able to contribute little more as held by Henderson (5). These records also corroborate an observation of Gesell (9) viz., that to be effective, the A_s-V_s interval must not be too long. Frequently auricular systole apparently injects a considerable volume during the dynamic phase of auricular systole but if the A_s-V_s interval is sufficiently long the curve returns to its previous level indicating a back flow of blood toward the auricle (fig. 6, *A*). In such cases auricular systole cannot be said to aid ventricular filling. If this interval is shorter time for this back flow of blood is lacking and the contribution of auricular systole is permanent (e.g., fig. 6, *B*). On the whole, however, our records show that even in slower beats the auricle contributes more than Henderson has generally held.

Such a critical study of volume curves shows that while they are subject to distortion, due largely to position changes of the heart, it is possible to discount these artefacts because they occur only in those portions of the records which come during the very early moments of ejection and during the early diastolic interval when no actual volume changes are taking place. The systolic and diastolic portions of the records which we consider reliable are indicated in figure 2, by the lines *P Q* and *R S*, respectively.

METHODS OF EXPERIMENTATION AND PROCEDURE FOLLOWED IN ANALYSIS OF RESULTS. In order to study the separate effects of changes in venous return, arterial resistance and heart rate in the intact circulation, the procedure recently described by one of us (3) was followed. At appropriate intervals, during which artificial respiration was temporarily discontinued, optical volume and pressure curves were simultaneously recorded. During venous infusion, such tracings were taken with every increase in right auricular pressures of 10 mm. Venous pressures were measured according to the principle suggested by Henderson (5) but the apparatus was adapted to use in connection with saline infusion. Arterial resistance changes were induced chiefly by partial or total compression of the aorta just above the

diaphragm, although reflex vasoconstriction was also studied. Optical records were taken during the acts of compression and decompression (immediate effects) and at various intervals thereafter (stabilized effects). To avoid heart rate changes, the vagi nerves were divided except when specifically noted. Heart rate changes were induced by cutting and stimulation of the vagi, by asphyxia and small doses of epinephrin, optical records being taken in all cases to show immediate and stabilized effects.

THE VOLUME CHANGES DURING INCREASED VENOUS RETURN. *Previous work.* The results of Patterson, Piper and Starling (8) obtained

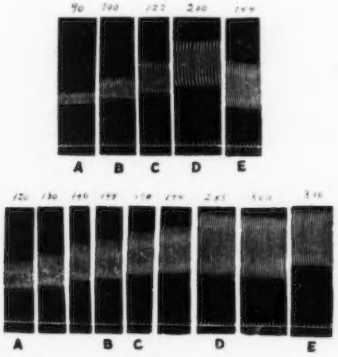

Fig. 4. Two series of segments from continued drum tracings of ventricular volume curves taken during progressive increase of venous pressures due to saline infusion. Experiment C 289 (⅓ actual size). Numerals above segments indicate right auricular pressure readings in millimeters saline. Downstroke systole.

Upper series, effects when heart slows (vagi undivided). *E*, Effect of vagus section.

Lower series, effects when no change in heart rate occurs. Letters below segments correspond to similar letters in figures 5 and 7.

on a heart-lung preparation, indicated that both systolic and diastolic volumes of the ventricles increase, and that the systolic discharge becomes larger when the venous inflow is augmented. Straub (11) confirmed these observations except that he noted no alteration in systolic volume.

According to Patterson, Piper and Starling (8), the systolic portion of the curve changes markedly in contour. Their curves, which at low venous pressures were divisible into three phases (rapid ejection, retarded outflow and secondary increased ejection), became smooth and

steeper as venous pressure increased. Straub, in an earlier paper (11), noted an increased steepness of the latter part of ejection as the only effect. In curves derived from integration of tachygrams (10) he subsequently found a steeper gradient in all phases of ejection. Both observers found a more rapid diastolic filling, Straub mentioning, however, that in all cases auricular systole contributes about 60 per cent to total ventricular filling. No changes in the duration of systole were noted by Patterson, Piper and Starling and a slight increase by Straub.

Our results. The typical effects of increasing venous pressure on the volume curves recorded on slowly moving paper are illustrated in figure 4. In the experiment shown in the upper series, the vagi nerves

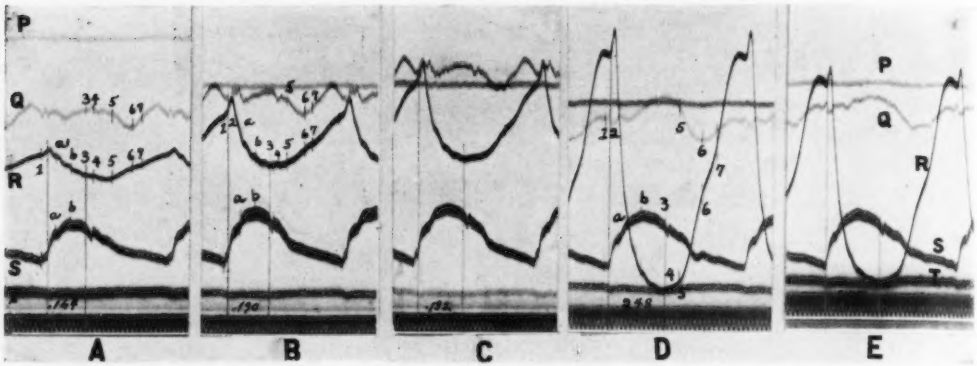

Fig. 5. Five segments of optical volume curves, *R*, left auricular pressures, *Q*, aortic pressure, *S*, from experiment C 289 taken under different venous pressures (½ actual size). Letters correspond to similar letters in lower series of figure 4. *P*, base line for left auricular pressure; *T*, base line for aortic pressure. Time is 0.02 second. Numerals same as in figure 2.

Observe progressive increase in duration of ejection phase (figures above tuning fork), increased steepness of both systolic ejection and diastolic filling curves, changes in left auricular and aortic pressures. Further description in text.

were uncut, consequently the heart slowed and venous pressures mounted rapidly. In the lower series the vagi nerves had been cut; the cardiac cycle was practically unaffected and venous pressure rose slowly. Such records confirm, in the main, the results obtained by Starling and his collaborators. They clearly demonstrate that the systolic discharge increases progressively as venous pressures are in-

creased step by step to levels far exceeding that set by Henderson and his collaborators (5) as "critical." The limit of response was not reached until pressures ranging in various animals from 250 to 310 mm. were attained. Above these levels decompensation and decreased discharge occurred. As the venous pressure increased, the ventricles dilated. The diastolic volume was affected more than the systolic, accounting for the increased stroke. These changes occurred regardless of whether aortic diastolic pressure remained unaltered or was somewhat increased.

The diastolic dilatation was more pronounced when the heart coincidently slowed (cf. fig. 4). As shown by comparing segments *D* and *E*

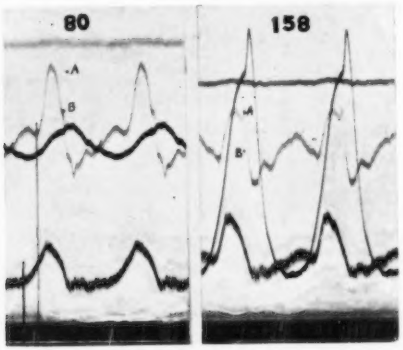

Fig. 6. Two original optical volume curves together with left (upper) and right (lower) auricular pressures from experiment C 290 (½ actual size). Venous pressure in first segment equals 80 mm. saline, in second = 158 mm. Complete series transcribed in figure 7. *A*, *B*, corresponding points showing elevation of initial pressures in left heart. Note precedence of right auricular over left auricular wave, also decrease in As–Vs interval after infusion.

of the upper series, this slowing contributed not only to the diastolic distention but to the venous pressure. Both were greatly reduced when the slowing effect was abrogated by vagal section. When no change in heart rate occurred (lower series) systolic discharge increased until a pressure of 285 mm. supervened, remained unchanged as it climbed to 300 mm. and was actually reduced when the level of 310 mm. was reached.

The cause of such an increased systolic discharge may be studied in original optical or transcribed curves taken during such experiments,

(cf. figs. 5, 6, 7). They show that the increased systolic discharge was accomplished in part through an increase in velocity of ejection, in part, however, by a prolongation of systole. When an increase in the velocity of ejection occurred, it affected not only the earlier phase of maximum ejection *2-a*, but also the gradient of the reduced ejection phase, *b-3*. This is clearly shown in the original segments of figure 5. These changes in the details of ejection are reflected both in the pulmonary and aortic pressure curves. In both circuits the pulse pressure increased, the gradient of primary rise was steeper and systolic ejection was prolonged, the maximum ejection phase being especially increased. Incidentally, such curves as figure 5 indicate no elevation of diastolic pressure, in fact during the higher infusions it began to decrease slightly, thereby demonstrating clearly that it is quite possible in intact animalls to vary venous pressures without affecting heart rate or arteria resistance.

A comparison of curves (fig. 7) as regards the superimposability of their systolic portions shows that especially in the lower ranges of venous pressures, the systolic strokes do not coincide but become steeper. In the higher ranges (e.g., 160 to 300 mm. in *C 288, IX*, fig. 7) the gradient often remained the same, making the systolic strokes quite coincident. While in such cases, the systolic strokes at the lower pressures are segments of the more extended strokes at higher levels, the changing length of systole is in no sense a function of diastolic length but of the rate of venous filling.

Is the increase in duration of systolic ejection due to a prolongation of the contractile state or to an earlier incidence of systole? We have measured the A_s-V_s intervals in several original records such as are illustrated in figure 5 and found that, as a rule, this interval was not decreased. Occasionally, however, (fig. 6) it shortened by 0.02 second. Our observations of such changes are not extensive enough, however, to say whether it was more than a chance variation.

With diastole unchanged (*C290*, fig. 7) or even shortened during infusion (*C 288*, fig. 7), it is obvious that the greater discharge must be due to an increased rate of filling. All transcribed as well as original records show that this was the case. At no two venous pressures above 60 mm. were the diastolic portions of the volume curves superimposable. The increased rate of diastolic filling took place irrespective of whether or not the dominant heart cycle included a diastasis phase.

A study of the separate filling phases of diastole (viz., early diastolic inflow, *5-6*, diastasis, *6-7*, or auricular systole, *7-1*), shows that each

phase shared in the greater inflow. The diastasis flow which was very gradual at venous pressures near 60 mm. became progressively steeper as venous pressures mounted, and a casual inspection of drum records gives the impression that the rapid inflow continued uninterruptedly throughout diastole. Careful inspection of optical tracings shows a distinct change in gradient (*C 288, IX* fig. 7) at the beginning of diastasis.

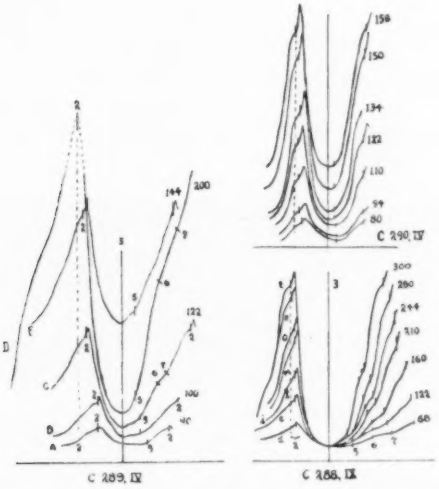

Fig. 7. Three series of transcribed curves showing prolongation of systolic ejection, increased velocity of ejection through increasing steepness of gradient and alterations of diastolic filling as venous pressures increase (⅓ actual size).

C 288, IX. Curves matched at end of systole without regard to actual volume in order to show degree of superimposability of systolic and diastolic portions. Letters refer to original records; numerals, to right auricular pressures in millimeters saline; other figures as in figure 2.

C 289, IV. Curves from same experiment as upper series in figure 4 plotted according to actual systolic and diastolic volumes. Capital letters correspond to those of figure 4.

C 290. Superimposed curves showing same changes as in *C 288* without change in heart rate. Lettering same.

As regards the relative filling during the early diastolic inflow phase and during auricular systole, actual computations showed that each phase shared about equally in the increase. Thus, actual calculations of curves *D* and *R* in experiment *C 288*, figure 7 showed that the auricu-

lar systole contribution increased from 50 to 54 per cent, while the early diastolic inflow increased from 30 to 36 per cent of the total filling. The greater rate of inflow during the early diastolic inflow phase was undoubtedly due to the greater venous pressures, shown in figures 5 and 6 to occur at the moment of opening of the a-v valves.

It was pointed out before that, whenever the heart slowed during an infusion, the diastolic volume increased more rapidly than when the rate remained the same. This additional increase shown in experiment *C 289, IV*, figure 7, was due to the relatively large inflow which obtained during the diastasis phase, *6-7*, under high venous pressures.

We may conclude that the greater diastolic inflow is due *a*, in rapidly beating hearts, to an increased rate of filling during both the early inflow phase and during the auricular systole phase; *b*, in slowly beating

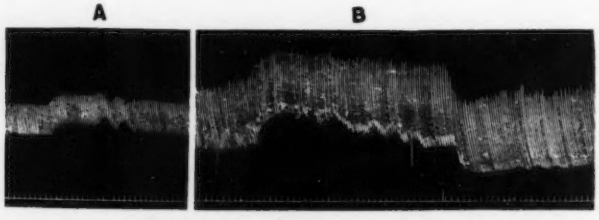

Fig. 8. Two segments of drum records from experiment C 295, VI and XIII, showing the general reaction of the heart to aortic compression and decompression. In *A*, right auricular rose from 92 to 98 mm.; in *B*, from 132 to 160 mm. saline (½ actual size). Downstroke, systole.

hearts having a diastasis phase of some duration, to an additional increase in rate of filling during diastasis as well.

Having pointed out the mechanisms through which it is possible for the heart to increase its systolic discharge, a discussion of the fundamental cause of such augmented discharge still remains. Obviously, the diastolic volume and initial length increase. Patterson, Piper and Starling found this not necessarily associated with an increase in ventricular pressure. Straub, however, noted a definite increase in initial right ventricular pressure. Experiments recently reported by one of us (3), (13) indicated that the increase in initial tension occurred in both ventricles and that changes in initial volume were never dissociated from changes in tension. A study of the right and left auricular pressures recorded simultaneously with volume curves enables us to judge of coincident changes in initial intraventricular tensions for, at

the moment when ventricular systole begins, the pressures in auricles and ventricles are undoubtedly equal. Such curves (figs. 5 and 6) show clearly that, up to a certain point, the pressures at this moment increase in both auricles as the ventricles distend.

It has been previously pointed out that somewhere between 250 and 300 mm. of saline a critical pressure level is reached at which the discharge decreases and the heart decompensates. Several causes for such decompensation may exist. First, however, an artificial cause of failure must be discounted. As the heart dilates more and more it may happen that the cardiometer rubber or even the cardiometer itself compresses the heart during diastole and thus retards its filling. Such a condition can readily be recognized by the flat contours of the diastolic filling curves. Failure of the heart may occur through the supervention of a mitral or tricuspal regurgitation but is generally due to a failure of the right ventricle. Such decompensating changes are well shown in the last two segments of experiment *C 283*. At venous pressures of 310 mm., we note that the amplitude of the volume curves has decreased (fig. 5) and, in the graphic records (fig. 4), we observe the beginning of pathological dilatation leading rapidly to complete decompensation. A closer study of the optical volume and pressure curves (fig. 5) indicates, however, that the process of decompensation had begun before—that it was inaugurated in fact while the joint volumes ejected by the two ventricles ostensibly continued to increase, e.g., at venous pressure of 285 mm. (fig. 5, *D*). We note in this segment that not only had the left auricular pressure been reduced but the systolic and diastolic aortic pressures were lower. This can only be interpreted as indicating a reduction of the left ventricular discharge. The fact, however, that left auricular pressures were reduced while right auricular pressures continued to mount indicates that primarily the right ventricle must have failed to supply the left auricle with a volume corresponding to the increased inflow into the right auricle. Hence, while the joint volumes ejected by the two ventricles still increased, the right ventricle was unable to remove effectively such volumes as accumulated in the right auricle and its supplying veins.

THE EFFECTS OF INCREASED RESISTANCE: *Previous results.* Patterson, Piper and Starling found that when the arterial resistance was suddenly increased to a great degree, both systolic and diastolic volumes of the ventricles increased approximately equally. If anything, the systolic discharge augmented slightly. Venous pressures increased coincidently so that their results represent the combined

effects of an increase in venous pressure and arterial resistance. Each heart, as these investigators found, has a limit beyond which it no longer responds to increased resistance but dilates further and decompensates. The resistance level at which such decompensation occurred was found to be lower when the venous inflow was great than when it was moderate. Similar results were reported by Straub (11) when the resistance was gradually increased step by step.

In studying the changes in the contour of the volume curves, Patterson, Piper and Starling found changes in the systolic slope, smilar to those already analyzed as effects of increased venous return. They also noted a more rapid diastolic filling rate due to the increased venous pressures and presented optical curves which indicate a definite prolongation of systole. Straub, in an earlier paper (11), observed no changes which he ventured to interpret as showing noteworthy deviations in contour. In curves reconstructed from tachygrams subsequently reported (10), he found that the steepness of the entire ejection curve increased and the amplitude was greater. This he attributed, not to a direct effect of the increased resistance, but to an indirect effect produced by the increased coronary blood flow. Straub says that the diastolic portions of the volume curves were not affected but to us the curves themselves appear steeper indicating with the ventricular pressure curves themselves appear steeper indicating with the tension was greater.

Our results. In accord with the results obtained by the aforementioned investigators on a "heart-lung preparation," we also found in the intact animal that any increase in arterial resistance, whether moderate or great, gradual or sudden, always caused, after a few beats, an increase in both systolic and diastolic volumes, the systolic discharge remaining unaltered or slightly increased, provided of course that the heart was in good condition (fig. 8). Unless the heart slowed, however, (vagi intact) we never observed such an extreme dilatation as these investigators. Thus, with complete compression of the aorta above the diaphragm we never observed an instance in which the cardiac volume in systole was as great as during diastole before compression. Neither was the venous pressure elevated as much as shown in their results. In the curve illustrated in figure 8, *A*, the mean arterial pressure rose from 50 to 100 mm. Hg., right auricular pressure elevated from 92 to 96 mm. saline. In such cases it was possible to study the sole effects of augmented resistance in the intact circulation under conditions that were apparently better than in the isolated heart.

Under such conditions of augmented resistance, the duration of systole invariably shortened (fig. 9). This was not a chance variation, inasmuch as it could be reduplicated many times in each experiment. It occurred with remarkable promptness when compression was sudden, being evident during the first cycle following such compression, (fig. 9, *A*) and progressively decreased during the existence of compression, *B*. Upon decompression, *C*, the systolic ejection phase lengthened again.

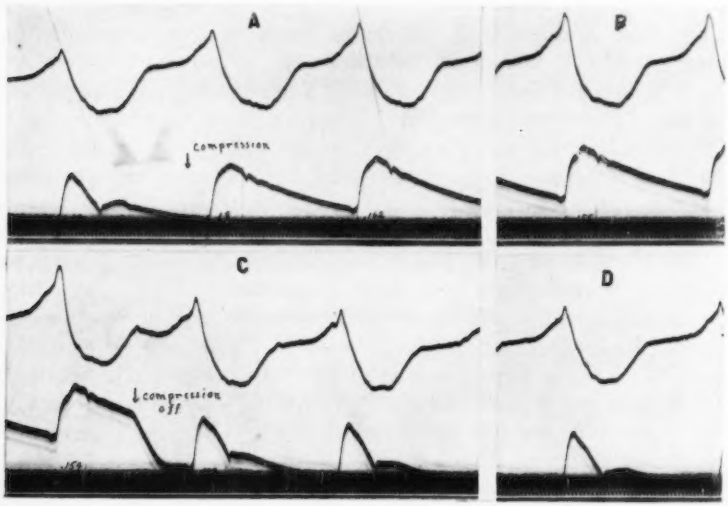

Fig. 9. Four segments of optical volume curves and aortic pressure curves from experiment C 295, VII, in which heart was moderately slowed by continued mild vagus stimulation in order to bring out all diastolic phases. (⅓ actual size). Drum record was similar to first record of figure 8, *A*.

A: Effect of aortic compression showing prompt abbreviation of systole, steeper ejection gradient, very little change in diastolic volume and diastolic filling.

B: Stabilized effect, 10th beat after.

C: Effect of decompression showing volume curve distortion by sudden decrease of pressure, reverse changes in contour of systolic ejection, lengthening of this phase, etc.

D: Stabilized after-effects.

The cause of the shortened systole is also indicated in this record, as well as in the transcribed curves of figure 11, *A*, *C*. It is obvious

that in such cases the shortening of systolic ejection is accomplished by a steeper ejection gradient particularly in the earlier phase of ejection. In other words, the ventricles eject the same or even a greater discharge in a shorter interval. This we believe to be the mechanism by which a good heart responds to increased resistance. When hearts are not capable of responding in this way, the output is reduced, for, when the phase of systolic ejection is reduced and the gradient of systolic ejection is not changed no other event can follow. Indeed it seems that the ability or inability of the ventricles to respond to increased resistance with increased velocity of expulsion determines whether the systolic discharge is augmented, unaltered or decreased. In the normal, as well as in the hypodynamic heart, the phase of systolic ejection is reduced. The former is able to respond (independent of venous pressure changes) by a greater velocity of ejection so that the same or even a greater volume can be expelled in less time; the latter expels blood at the same velocity with the result that the systolic discharge is decreased and diastolic dilatation becomes greater.

When venous pressures rise either because experimental hearts are somewhat hypodynamic (i.e., react by smaller strokes) or have been distended by higher venous pressures (after or during infusion) the systolic shortening is only temporary, the ejection gradually increasing again to normal. This is well illustrated in figure 10, representing a record from the experiment shown in figure 8, *B*. In this case, a slow saline infusion was continued throughout the compression and venous pressures rose from 108 to 130 during compression. Obviously initial volume and presumably the initial intraventricular pressures augmented at once, to a certain degree. Nevertheless systolic ejection was reduced in the early beats from 0.16 to 0.14 second and the systolic discharge was greatly augmented only and solely because of a steeper ejection. As venous and initial volumes progressively increased during the course of this observation, it was found that the interval of systolic ejection gradually lengthened, however, until the normal figure of 0.16 second was reached again. Such lengthening may only be attributed to the coincident increase in venous pressure, for in eleven previous trials in this animal in which no such elevation of venous pressure occurred, systolic ejection was always permanently reduced on increasing the resistance and lengthened when resistance was again reduced.

These observations substantiate the suggestion recently made by one of us (3) that the increased duration of systole found by Patterson, Piper and Starling was due to the fact that coincident changes in venous pressure obtained in their experiments. In other words, a lengthening of systole accompanying increased resistance is not a direct reaction of the heart to such changes as these investigators maintain but the reaction to predominating changes in venous pressures which may attend it.

As regards the superimposability of the volume curves, it was found and is illustrated by figures 9, 10 and 11, that the diastolic portion may

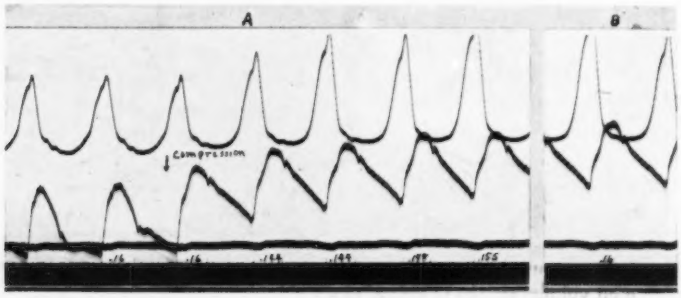

Fig. 10. Effect of compressing aorta during continued venous infusion comparable to second curve of figure 8.

A: Immediate effect. Observe primary shortening of systolic ejection phase, steeper ejection rate, increased diastolic filling rate. Later, gradual lengthening of systolic ejection as right auricular pressures increased from 144 to 180 mm. saline.

B: Stabilized effect—right auricular pressure, 190 mm. saline. Duration of systolic ejection returned to normal but curves not superimposable with same.

or may not be superimposable, depending solely on the changes in venous pressure attending these changes. When venous pressure was unaltered, the curves remained superimposable; if it rose the same changes as discussed in a previous section obtained. Since systolic ejection occurs more steeply in a vigorous heart, this portion of the curve was not superimposable especially as regards the early phase but nowhere are such marked deviations shown as in the cases where venous pressure increased. When the output was decreased in hypodynamic hearts, the early phase was superimposable but ejection became more gradual during the later portion of ejection.

These detailed changes in contour during increased resistance agree quite well with the interpretations of Straub but are not in accord with the findings of Patterson, Piper and Starling. The latter investigators record changes which indicate that during compression the curves become flatter and that there is a late systolic phase during which no ejection takes place. Such a tendency is shown in a few of our curves but, in these instances, as previously pointed out, the flattened portion of the curve is a post-systolic and not a late systolic phase. Only once did we find such changes actually occurring during systolic ejection. Before compression, the systolic ejection was definitely divisible into three parts: a phase of rapid expulsion, a phase

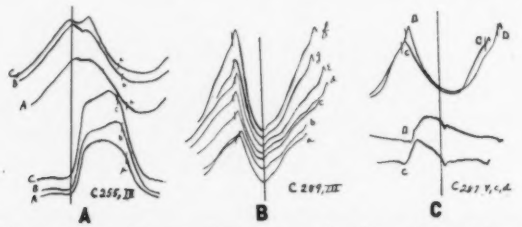

Fig. 11. Three series of transcribed curves showing effect of increased resistance on volume curves (⅓ actual size).

A: Simultaneous volume and intraventricular pressure curves, matched at beginning of isometric phase and showing reduction of systole and increased steepness of curve, A, normal, B and C, during compression.

B: Series of tracings before, during and after compression when venous pressure increased—every third beat redrawn; *a*, normal; *b*, third beat after compression showing shortening of systolic ejection; *c*, *d* and *e*, etc., showing progressive lengthening as venous pressure increases and heart slows.

C: Two records of simultaneous volume and aortic pressure curves showing abbreviation of systole and increased steepness of ejection with little effect on venous pressure and initial volume in spite of slight lengthening of cardiac cycle. *C*, normal; *D*, after compression.

of halted ejection and a final phase of accelerated ejection. Furthermore these phases disappeared during compression and the ejection curve as a whole was smoother. A careful comparison of the volume and pressure curves before compression clearly showed, however, that such volume curves were not reliable, for the apparent decrease in discharge occurred precisely where the aortic pressure mounted and the late increase corresponded exactly with the moment when the aortic pressure began to decline. Such discrepancies can only be interpreted to mean that the volume curves were inaccurate.

When the heart rate slows coincidently with the elevation of aortic pressure (vagi intact) and, as a result, the venous pressure rises, these influences tend to increase the duration of systole and thus may diminish, neutralize or overbalance the tendency of increased resistance to shorten the contraction process. The curves of figure 11, *B*, show such combined effects. Curve *a* is normal; *c*, *d*, *e*, represent every 3rd volume record following compression. We observe, in the first beat, a tendency for systole to shorten but as the heart slowed and diastolic volume increased in successive beats, systole lengthened. That even here there is a tendency to abbreviate systole is shown by the fact that we do not observe the systolic lengthening which ordinarily accompanies such slowing.

CONTOURS OF VOLUME CURVES DURING HEART RATE CHANGES. Since we found no definite relation between the lengths of consecutive systoles and their preceding diastoles during the course of heart rate changes, we concluded in previous papers (1), (2), (3) that this must indicate that the ventricular volume curves can not be superimposable under such conditions. Among other observations, we pointed out *a*, that, during moderate cardiac slowing induced by direct vagus stimulation, the phases of systolic ejection progressively increase without relation to the lengths of previous diastole; *b*, that, during cardiac slowing produced by hypercapnia (partial asphyxiation), the phase of systolic ejection either might not change at all or become shortened; *c*, that small doses of epinephrin (accelerator stimulation) always cause a definite abbreviation of the ejection phase regardless of whether the heart accelerates (vagi cut) or retards (vagi intact). These facts were substantiated in this series of experiments in which the nature and causes of the volume changes could be definitely followed.

Types of reaction during the course of direct vagus stimulation. At least two types of reactions, illustrated by the slow drum records of figure 12, *A* and *B*, were obtained. In the type of reaction illustrated in *A* the heart distended during diastole, and, while it emptied itself with normal completeness during the first few beats, there was a gradual tendency to empty less and less, thereby progressively diminishing the systolic discharge. In a few cases, this progressed so far that the systolic ejection actually became less than during the more rapid normal beats. This effect was observed while venous pressures ranged from 70 to 150 mm. but in all cases arterial pressures were either relatively low at the start or fell to low levels as a result of vagus

stimulation. Probably this type of reaction supervenes therefore whenever the coronary blood supply is reduced below a level which may be regarded as minimal for efficient cardiac contraction.

When arterial pressures fell less or were prevented from dropping to any marked degree by partial compression of the thoracic aorta previous to stimulation, the type of reaction illustrated in *B* was obtained. These curves differ in that systolic discharge continued as complete as before and that, in consequence, curves of larger amplitude consistently occurred during vagus stimulation. When inscribed on more rapidly moving drums, the curves at different rates show a fair degree of superimposability, when redrawn without regard to the exact end of systole. We believe therefore that this type of reaction, obtained when coronary blood supply remains adequate, would be regarded as a typical and standard reaction by Henderson and his collaborators.

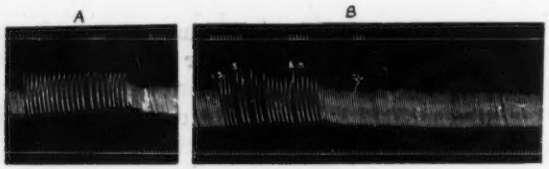

Fig. 12. Two segments of drum records illustrating two types of vagus slowing following direct vagus stimulation. Detailed description and reference to numerals on records in text. (⅓ actual size.) Systole, downstroke.

In both types of reactions, however, the systolic ejection phase lengthened in successive beats until stabilized conditions were attained. Thus in the case of experiment *C 289* illustrated in figure 12, *B*, the numbered beats had systolic ejection phases of the following order: (1) 20.5, (2) 0.24, (3) 0.25, (4) 0.25, (5) 0.32, (15) 0.29, (17) 29+, (36) 24.5. The obvious lack of relation to previous diastole lengths can readily be verified by comparing the paired beats with equal cycle lengths (e.g., 1 and 36, 2 and 17, 5 and 15). This can only mean that the volume curves are not superimposable.

Changes in the systolic portion of volume curves during vagal slowing. The immediate and stabilized changes in the volume curves during direct vagus stimulation are shown in original and transcribed optical volume curves of figures 13 and 15. We note immediately after the inauguration of stimulation and coincident with the increase in amplitude that the steepness of the systolic ejection gradient increased. In

successive beats following, the gradient became less and less steep, however, until its lower segment finally coincided fairly well with normal beats. The effect of this is that, while the systolic ejection phase is lengthened in the first few vagal beats, it is not prolonged to the extent that it would have been, had the curves continued superimposable. The transcribed curves of experiment *C 287* in figure 15 show especially well that the gradual decrease in velocity of ejection was the direct cause of the progressive lengthening of the systolic ejection phase before noted and that it prevented the curves from being superimposable either on each other or on normal curves.

What causes this progressive decrease in ejection rate and the consequent lengthening of systolic ejection? Since an increased venous pressure due to saline infusion was found to produce a lengthening of systolic ejection, and since, moreover, it was found that the systolic ejection lengthened as long as initial intraventricular pressure continued to increase, the suggestion was recently made by one (3) of us that the duration of consecutive vagal systoles is controlled by the initial venous pressure.

A careful study of these volume curves has shown, however, that while an increased venous pressure is indeed the cause of the initial lengthening of systolic ejection in the first few vagus beats, it is not the cause of the progressive prolongation of subsequent beats. Thus, in experiment *C 287* (fig. 15) the increased steepness and prolongation of the early beats (e.g., *1—4*) is undoubtedly due to increased venous pressure. It is obviously not the cause of the successively more gradual gradients indicated by *7* and *8* for, in these cases, both initial pressures and initial volumes were greater and, moreover, it has never been found that increased venous pressure tends to decrease the rate of ejection while this phase is lengthened; on the contrary the steepness always increases. In fact, considered from another angle, the increased rate of ejection during the earlier beats tends to make the phase of systolic ejection shorter than would have been the case, had the curves continued superimposable with the normal.

The explanation of the more gradual ejection which may even lead to smaller and smaller amplitudes when arterial pressures are low must be sought for in other directions. Since a slower ejection rate occurs when arterial resistance is reduced, it might be supposed that this predominates over the increased venous pressure during vagus stimulation. Such an interpretation might be given to many records in which arterial pressures fall considerably. It could hardly have

been a factor in the experiment from which curves *C 287, I* or *C 289* were taken for, in the former, the entire fall of diastolic pressure amounted only to 4.5 mm., while in the optical curves of *C 289* it is practically equal; yet marked variations in rate of ejection are obvious. No more than a speculative hypothesis may be advanced to explain such cases. The possibility of course exists that the stimulation of coronary vasomotor fibers in the vagus (14) causes sufficient reduction of ventricular blood supply to affect the rate of contraction. Furthermore, in spite of much evidence to the contrary, the possibility should

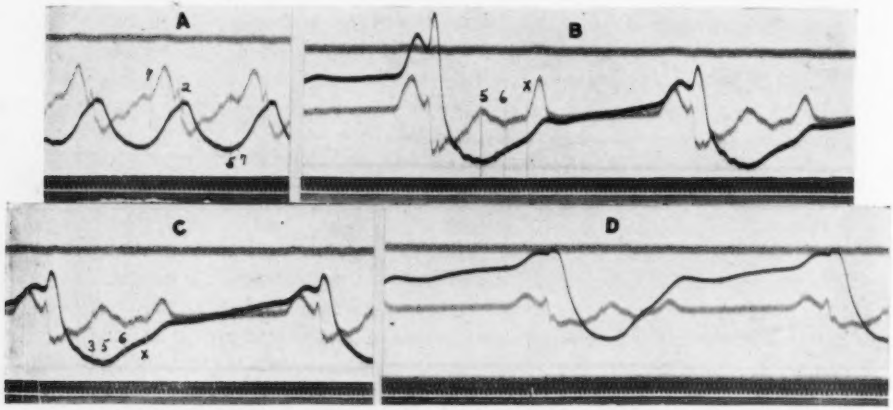

Fig. 13. Four segments of optical curves and left auricular pressure curves from experiment C 290, I. (½ actual size). *A*, normal beats; *B*, first and second vagus beats; *C*, fourth and fifth vagus beats; *D*, stabilized vagus beats. Observe changes in contour in various beats, effects of auricular systole of gradually diminishing vigor, less deformation of curve in *D*. Small figures same as in figure 2. Further and detailed description in text. Note additional auricular systole at *x* in segment *B*.

be borne in mind that the vagi may exert some direct effect on the ventricles which develops progressively in its power as in the case of the auricles.

Diastolic contour changes during consecutive vagus beats. During vagus slowing, there is always some increase in venous pressure at the start. Nevertheless, as shown in the majority of curves, the rate of filling during the early diastolic filling phase is usually reduced at once (cf. fig. 15, *C 287, N* and *1*). This is undoubtedly due to the incidence

of auricular systole during this phase at rates which are normal for the dog and its delay to the end of a more or less extended diastasis phase in slowed beats. When auricular systole does not normally occur during this phase, the rate of inflow is distinctly augmented in the slowed beats as would be expected (cf. fig. 15, *C 292*, *C* with *A-B*). In neither event is this portion of the diastolic filling curve exactly superimposable. The gradients of the diastasis phases are frequently parallel, the only difference noted in many cases being that, as the venous pressure increases, the gradient begins at corresponding higher filling levels. When changes in venous pressure are more marked, the rate filling during early diastasis may also become steeper (e.g., fig. 15, *C 287*, *C 292*). When diastoles are unusually long and venous pressure is quite high, the phase of diastasis may be divided into two periods beginning as in *C 287* and *C 292* at x and y respectively. During the latter phase only may a condition of approximate stasis be said to exist. On account of these variations in venous pressure, the diastasis curve may not be regarded as strictly superimposable arcs of a standard curve.

The changes during auricular systole are well illustrated in figures 13 and 15. Thus in curve *N* of experiment *C 287*, figure 15, no distinct auricular contribution can be made out owing to the incidence of auricular systole early in diastole. During the first few vagal beats (e.g., 1–4) the auricular contribution is very much more marked than normal, and then decreases. Thus, we roughly estimate that the following percentage contribution is given to the ventricles by the auricle in various vagus beats: 1st, 40 per cent; 2nd, 36 per cent; 5th, 30 per cent; 8th, 22 per cent; stabilized 8 per cent.

This is in accord with observations described by one of us (15) and again clearly demonstrated in figure 13, viz., that owing to a prolonged diastole the auricular contraction first augments and the auricular wave of the intra-auricular pressure increases. Soon, however, the auricular contraction decreases in amplitude due to a specific effect of the vagus nerves. Then auricular contributions become progressively less and less until, during stabilized effects, very little contribution takes place. Owing to the varying amplitude of auricular contraction, this portion of the curve cannot be regarded as superimposable.

Summarizing, we may say that, since the ventricles do not necessarily empty to the same extent or at the same rate, since venous pressures usually are elevated, since auricular systole falls during different phases of diastasis and contributes variable amounts to ventricu-

lar filling owing to a direct influence of the vagi nerves on their contraction, the various portions of the filling curves are not superimposable during vagus slowing. It is this failure in superimposability which prevents the initial tension and initial volume of the ventricle from being determined by the duration of previous diastole and is furthermore the reason why, in general, duration of systole and amplitude of ejection are more definitely related to initial pressure and volume and not necessarily to duration of previous diastole.

Volume curves during hypercapnia (partial asphyxia). Since Henderson and his collaborators (5) produced changes in rate chiefly and preferably by varying the CO_2 content of the blood and since we have previously found that a frequent effect of such chemical slowing is the production of shorter systoles, the nature of the volume curves under such conditions was studied. We found a number of cases in

Fig. 14. Segment of drum record showing volume changes following ejection of 1:100,000 epinephrin solution when (vagi intact) it causes a primary acceleration and subsequent slowing. Right auricular pressure increased 30 mm. (⅓ actual size.)

which systole was unaltered but only two cases in which a sight prolongation occurred during very slow beats. In the great majority of instances, we noted a decrease in systole during the slowing phases and a still further shortening during recovery (fig. 16). In spite of the tremendous increase in initial venous pressure and initial volume, together with a much increased discharge shown in experiment *C 247*, the duration of systole was decreased. Quite obviously neither the systolic nor diastolic portions of the curve were superimposable in any sense, but became more rapid during and after asphyxial slowing.

What factor prevents the increased venous pressure from lengthening systole by so greatly increasing the velocity of discharge? Why does the rate of ejection not progressively increase as in the case of vagus stimulation? Again we are forced to probable rather than quite certain explanations. Since asphyxia is accompanied by marked

elevation of arterial pressures, the shortening influence of increased resistance was recently suggested by one of us (3) as a probable agency at work. That it explains many volume curves quite satisfactorily and in many cases is a factor concerned must be admitted. We meet instances, however, as in figure 16, *C 289, I*, in which, owing to great retardation, diastolic pressure was actually lower than normal in beat *B* and still with the great slowing and increased venous pressure systolic ejection was somewhat abbreviated. We are therefore forced to consider the possibility that the accumulation of CO_2 affects the accelerator mechanism or heart itself, either directly or indirectly by augmenting the output of epinephrin. At all events some other factor is brought into play which determines the abbreviation of systole and improves the ejection of the heart so as not to affect the systolic discharge.

The contour of volume curves during action of epinephrin. Our previous observations that epinephrin consistently caused a relative shortening of systolic ejection regardless of whether the heart rate increased or decreased was confirmed in these experiments on smoked drums. Epinephrin caused, especially when no great acceleration was induced, an increased diastolic distention and a larger systolic ejection. When the heart slowed systolic emptying was more complete, an effect which persisted for some time (fig. 14). Venous pressures were always increased at least 20 to 30 mm. even when heart rate accelerated. Optical curves illustrating the changes in contour are shown in figure 15, experiment *292*, where the changes may be compared not only with cycles normal to the animal *C*, but with stabilized vagal beats as well, *A-B*. We note, on comparing the epinephrin beats *D-E*, that the duration of systolic ejection was decreased by virtue of a pronounced increase in the ejection rate. Obviously, the systolic ejection curve is superimposable neither on normal nor stabilized vagal beats. We have previously presented evidence to show that the great reduction of systolic ejection thus produced, in spite of a contrary tendency of increased venous pressure to lengthen it, is not entirely determined by the augmented arterial resistance. This was again corroborated and is illustrated in experiment *C 292* shown in figure 15. Here the diastolic pressure was not appreciably increased over normal in beat *D* yet a pronounced alteration of systolic ejection obtained. We are thus again forced to assume a specific effect on the cardiac or accelerator mechanism. Such curves also show that the diastolic filling curves are not superimposable. This is due to several easily recognized causes: Epinephrin increases the venous pressures

which, as is especially well brought out in comparing curves *C* with *D* and *E*, causes a more rapid filling during both the early diastolic inflow and diastasis phases. In addition, however, complete filling which determines both initial ventricular volume and initial pressure and through these the amplitude of systolic discharge is materially aided by augmented auricular contractions. This much greater actual increase is clearly illustrated by comparing the auricular increment in beats *A* and *C* with *D* and *E*. Here calculations show that the percentile increase is also much increased. Thus, while auricular systole roughly contributed about 8 per cent to the total filling during stabilized vagus beats, *A*, and 33 per cent to total filling in the normal beats, *C*, it increased to 44 per cent during the epinephrin beats *D-E*.

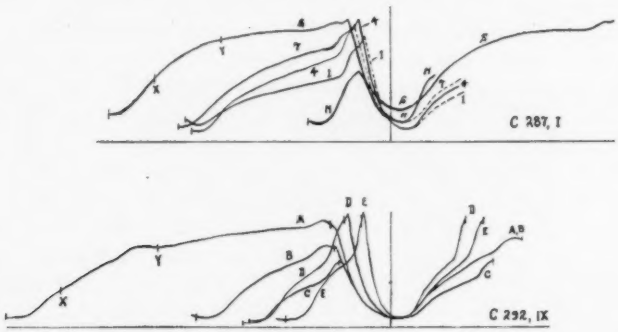

Fig. 15. Two sets of transcribed curves showing lack of superimposability at different heart rates. (⅓ actual size.)

C 287. Series of vagus beats showing immediate and stabilized effects on volume curves, *N*, normal. *1*, *4* and *7* represent first, fourth and seventh vagus beats. *S*, stabilized vagus beats. Curves matched at end of systole. Note: failure of superimposability in all vagus beats with normal and each other; progressive lengthening of systolic ejection due to more gradual gradient; progressively diminishing auricular contribution; increasing inflow during early diastole and diastasis due to augmenting venous pressures.

C 292, IX. Series of beats at different heart rates. *A*, *B*, stabilized vagus beats with different diastolic lengths. *C*, normal; *D*, early epinephrin action producing slight acceleration; *E*, after effect of epinephrin. Note failure of epinephrin curves to superimpose even approximately on stabilized vagus and normal beats as regards systolic or diastolic portions, also extreme abbreviation of systolic ejection phase with steeper systolic strokes. Note only approximate superimposability of normal curves *C*, with stabilized vagus curves *B* and *A*. Note increased auricular contribution during epinephrin and decreased contribution of stabilized vagus as compared with normal, *C*.

The determining influence of previous diastolic length during heart rate changes. The demonstration that the duration as well as volume of systolic ejection is dependent to so great a degree on such factors as venous pressure, arterial resistance, and to specific effects of nerves and chemicals on the heart and its vascular supply, does not carry with it the implication that the length of previous diastole is in no way concerned. In previous communications we have always emphasized not only that this mechanism must always be operative, but have pointed out its importance in the control of both the duration and length of systolic ejection when other conditions were not greatly

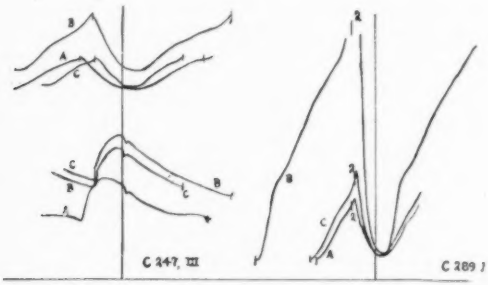

Fig. 16. Two series of transcribed optical volume curves showing failure of superimposability when heart rate changes are induced by asphyxia. (½ actual size.)

C 247, III. Effect of rate changes induced by asphyxia; *A*, normal; *B*, asphyxial slowing with steeper ejection and shortened systole; *C*, accelerated beats following restoration of artificial respiration.

C 289, I. Pronounced slowing during asphyxia in dog showing tremendous increase in systolic discharge, increased velocity of systolic ejection, shortened systolic ejection phase, in spite of pronounced lengthening of cycle.

affected. That under such conditions the beats are practically superimposable has always been admitted and is again beautifully demonstrated by comparing stabilized vagus beats of different lengths, *A* and *B* with normal *C* (fig. 15, *C 292*). Indeed, it was our original belief based on experiments of other researches that this was the chief mechanism controlling the duration and volume of systolic ejection under conditions approximating normal, and that the heart deviated from the principle only in specific cases, e.g., when the accelerator mechanism was involved or the heart was required to work under conditions of greatly changed arterial resistance and venous pressures.

The evidence presented in this and previous papers forces us to the conclusion, however, that even under quite normal conditions, changes in heart rate are not often *the factor* which dominates either the duration or the volume of systolic discharge.

SUMMARY

1. By the use of a segment recorder which, in a closed system, has a vibration frequency of 20 per second, it is possible to record directly and accurately such portions of the systolic ejection and diastolic filling curves as are required for study. We have found in physical tests that such a volume recorder faithfully records such changes as are produced in the cardiometer without altering normal cardiac action. While a number of incidents (e.g., bulging of valves, filling and emptying of coronaries, changes in volume of pulmonary conus and particularly the shifting of the beating heart into and out of the cardiometer) tend to distort the recorded volume curve, they may be satisfactorily discounted, inasmuch as they occur exclusively at times of the cardiac cycle when neither ventricular filling nor ejection take place (i.e., during the very beginnings of systole and diastole respectively). That the rates of ejection and filling are accurate, is further controlled by the fact that each change in ejection and filling rate corresponds precisely to changes in the aortic and auricular pressure curves.

2. As a result of our studies we interpret the following changes in ejection and filling rates:

In general, the rate of ejection is much greater during the phase designated by one of us as maximum ejection, and is suddenly much reduced during the phase of reduced ejection. During the phase of maximum ejection the rate of output is uniform, as Henderson and his collaborators maintain, only when arterial resistance is comparatively low. When it approaches or exceeds normal, the velocity of ejection changes in correspondence with optical aortic pressure curves. Usually, ejection is very rapid as the pressure curve ascends steeply, and becomes slightly less as the pressure summit is gradually reached, but there is no constant type of change that can be regarded as normal, for as many contour variations are found in the volume as in the pressure curves.

Diastolic filling under normal conditions of venous pressure we have found to proceed after the fashion described by Henderson and his collaborators. Early in diastole, a rapid inflow takes place which accounts for 30 to 50 per cent of the total filling. After this, the

inflow is retarded and diastasis supervenes. Contrary to Straub and Patterson, Piper and Starling, we have always observed a distinct phase of diastasis when the cycle is of sufficient length. While the volume contributed by the succeeding auricular systole varies under different conditions, our results indicate that on the whole it contributes more than the results of Henderson show, the volumes ranging from 18 to 60 per cent of total filling under normal conditions.

3. Studies of optical curves interpreted with the aid of simultaneous pressure curves show the following effects of increasing venous pressures by saline infusions:

Confirming the results of Patterson, Piper and Starling and contrary to the interpretation of Henderson and his co-workers, the ventricles respond with increasing strokes to increased venous return up to pressure levels far exceeding those set as "critical" by the latter.

Attending the increase in systolic discharge, we found, in accord with Patterson, Piper and Starling, an increase in diastolic volume and initial length, but, contrary to them and in accord with previous observations by one of us, such changes were never dissociated from changes in initial intraventricular pressures. Consequently, the statement that the only constant factor determining discharge is a change in initial length can not be regarded as demonstrated.

In accord with previous observations of one of us and contrary to results of Patterson, Piper and Starling, the duration of systolic ejection is progressively increased at constant heart rates, indeed, it is in part through such systolic lengthening that a greater discharge becomes possible. Prolongation of systole is therefore a direct function of initial volume and initial pressure.

Contrary to the law of superimposability, as stated by Henderson, neither the systolic ejection nor diastolic filling curves are superimposable during increased venous return but, corresponding somewhat to the theoretical curves drawn by Krogh, the rate of filling becomes more rapid in each of the consecutive phases of diastole and the velocity of ejection is also increased.

4. The following reactions of the ventricles to increased resistance were established:

Contrary to the results of Patterson, Piper and Starling and also those of Straub, and in accord with previous observations of one of us, the primary and constant effect of an increased arterial resistance is to reduce the duration of systole, not to prolong it.

In order to maintain a normal or augmented systolic discharge (which is the case in hearts which are in good condition) it is necessary for the velocity of ejection to increase. When the heart fails to respond in this way, systolic ejection is reduced.

The abbreviation of systole incident to augmented arterial resistance may be neutralized or overpowered when a considerable increase in venous pressure attends the elevation of arterial resistance (e.g., during coincident slowing or by decreased ventricular vigor in hypodynamic hearts). When no appreciable changes of venous pressure occur during increased arterial resistance, the diastolic portion of the volume curves is approximately superimposable, but the systolic strokes are never superimposable In the case of vigorous hearts the systolic gradient is steeper, in hypodynamic hearts the strokes may coincide with but are smaller than normal. Such beats are not to be regarded as superimposable in the sense of Henderson for the amplitude is not a function of heart rate.

5. A study of the slow beats immediately following vagus stimulation shows that the progressive increase in duration of systolic ejection phases is not associated with a progressive lengthening of previous diastoles. It is intimately associated with a progressive decrease in the ejection rate, i.e., a decrease in the gradient of the ejection curve. This, in turn, is not caused primarily by the gradually increasing venous and initial pressures as was previously suggested by one of us. In some instances it may be explained as an effect of reduced arterial resistance but, in other cases again, it is necessary to assume that some other factor operates (e.g., coronary vasomotor changes or specific effects on the ventricles).

Since venous pressure increases during the course of vagus stimulation, the rate of filling increases in all phases of diastole, with the exception of the phase of auricular contraction during which there is less and less contribution as stimulation continues.

Since the ventricles do not empty to the same extent or with the same velocity, and since the diastolic filling rates alter during vagus stimulation, neither the systolic nor diastolic portions of the volume curves are superimposable, either on normal curves or on each other.

The abbreviation of systolic ejection found during asphyxial vagal slowing or after epinephrin stimulation, is due to an increased velocity of ejection. This may be in part dependent on an increased arterial resistance but, in many cases, can be accounted for only by assuming a specific effect on the ventricles. If venous pressures are increased

to a marked degree during asphyxial slowing (but not during epinephrin slowing) this may tend to neutralize the effects and produce a shortening, in which case systolic ejection remains unaltered.

Owing to these marked deviations during asphyxial and epinephrin slowing, the volume curves are in no sense superimposable.

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ACTION CURRENTS IN STOMACH AND INTESTINE

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My first efforts to show the action currents in the bowel were made in 1913 when I was working in the Physiological Labratory at Harvard. At that time Dr. Alexander Forbes very kindly made a few records for me with the string galvanometer, just enough to show *a*, that currents of measurable intensity can be obtained from excised segments; and *b*, that the ordinary non-polarizable electrodes used in muscle and nerve studies are not convenient for work on the bowel.

In 1917 I again attacked the problem with the help of Dr. Robert Newell. We used segments of rabbits' small intestine contracting in a moist chamber. These segments were connected to an Einthoven galvanometer through zinc-zinc sulfate moist thread electrodes. A mechanical record was obtained at the same time by arranging a lever so that its shadow would be thrown across the slit of the camera. Although some good records were obtained, it was soon plain that few of the problems before us could be answered until better electrodes could be secured. The nature of the work demands first, that they be so designed that they can be attached quickly and easily in such inaccessible places as the cardiac end of the stomach (in the intact animal); second, that such attaching and detaching be done without trauma such as that involved in sewing on threads; third that the apparatus move with the viscus without sliding over its surface; fourth, that it work perfectly

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in any position; fifth, that it work under Locke's solution; and sixth, that it maintain a constant potential. Incidentally, I hoped to get an electrode which would not have to be made up fresh every few days. After experimenting during the last three years with almost every type of electrode heretofore described I have at last designed one which answers all the demands made upon it. As slight modifications are still being made from time to time, I will reserve a full description of the apparatus for a subsequent paper. Suffice it to say here that it is a small calomel electrode attached to a simple clamp.

Another problem at first was to get good contractions in the bowel without having it immersed in Locke's solution. The trouble is that the oxygenated bath which is so essential to the normal behavior, particularly of the excised gut, tends to short-circuit a considerable part of the action current which one hopes to detect with the galvanometer. Dittler (1), in his experiments on the crop of the sea-slug Aplysia, was able to avoid some of this shorting by lowering the conductivity of the solution. He got good results with an artificial sea water in which most of the NaCl was replaced with sufficient dextrose to supply the desired osmotic pressure. My own experiments with such a solution were not promising, but at that time I was using moist thread electrodes. With the new electrodes we have gotten good records even with the segments in Locke's solution. The excised bowel will contract rhythmically for some time in a warm moist chamber, but the conditions are abnormal; the amplitude of contraction rapidly diminishes; the rate becomes slow and irregular, and the action currents not infrequently become too weak to record (see fig. 1). I spent several months working with such segments in moist chambers without getting any really satisfactory results. Some of my difficulties were due to the fact that I was using rabbits, the intestines of which have thin walls, with little muscle and, naturally, weak currents. Better results were obtained when I began using segments from cats and dogs. As will be seen later, the best results can probably be gotten by using the intact bowel, with its circulation maintained.

Another difficulty with the gastro-intestinal action-currents is that while some of them are quite large, they tend to neutralize one another on account of the lack of synchronism in the muscular activity, i.e., one segment is relaxing while another is contracting. If the digestive tract as a unit were to pulsate regularly, or if a diastaltic wave were to shoot at short intervals from the cardia to the anus, the mass of muscle involved would be so large, compared with that in the heart,

that the record obtained by leading off from an arm and leg might be more of an electro-enterogram than an electro-cardiogram. As there is actually little synchronism of the movements in the various segments of the gut, we cannot expect to get good galvanometer records unless we place the electrodes close together on the viscus itself.

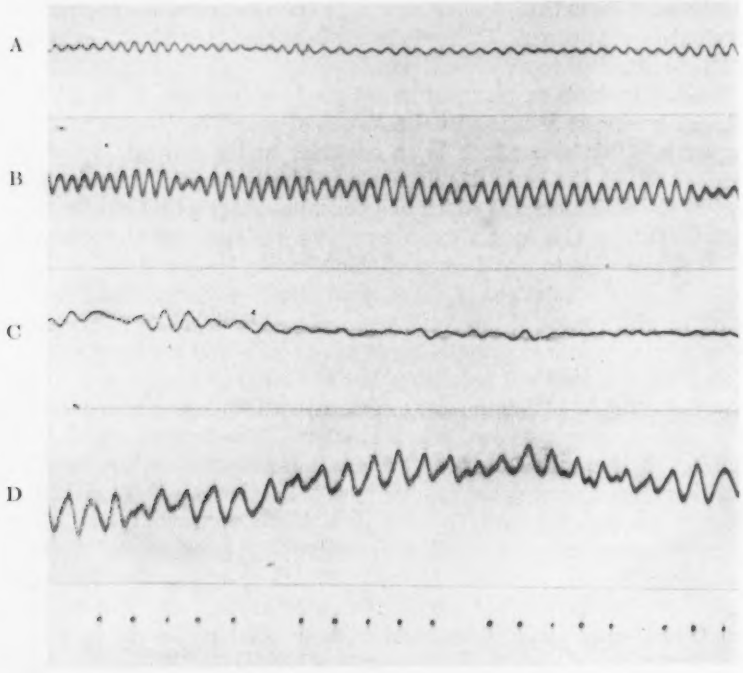

Fig. 1. Action currents from the excised duodenum, *A*, and jejunum, *B*, of the rabbit, with the gut in a bath of warm oxygenated Locke's solution (*A* and *B*); and out of the bath (*C* and *D*). The time record represents 10-second intervals with a double gap every minute.

Still another way in which I made things harder for myself at first was by using an Einthoven galvanometer. A good D'Arsonval instrument is more sensitive and better adapted for the recording of the slowly changing currents produced by smooth muscle. The disadvantage of the D'Arsonval is that the higher sensitivities are purchased at the price of increasing the weight of the moving coil, and lengthening

the period of its swing. I am now using two Leeds and Northrup instruments, one with a sensitivity such that 1 mm. deflection at a meter distance equals 3×10^{-10} amp., the other with a sensitivity of 6×10^{-10} amp. The first one has a period of 7.4 seconds, the second 5 seconds. The sensitivity is greater than necessary, and I have actually had to put in shunts at times to get rid of some of it, particularly when working on the antrum of the stomach. Some experiments done with an even more sensitive D'Arsonval instrument (5.37×10^{-11} amp.) show that the best galvanometer is probably the one just delicate enough to record the currents. Greater sensitivities bring with them all sorts of difficulties from stray currents, polarizations, differences in capacity, temperature, etc. The difference in sensitivity between an Einthoven and a good D'Arsonval galvanometer can be seen in the fact that in the standard electrocardiogram 1 cm. deflection represents 1 mv. while with the better of my two instruments 220 cm. represent 1 mv. when the camera is placed at a distance of 1 meter. We intend to use the Einthoven galvanometer hereafter only when the nature of the experiment requires the elimination of all deformity and time lag from the records.

During the last few months we have been getting very satisfactory records from the stomachs and intestines of dogs, cats and rabbits opened under ether or urethane anesthesia. The best incision to expose the stomach is a transverse one, running along the lower edge of the ribs from one side to the other. The cut is made between two clamps and the skin and peritoneum are then sewed together to stop hemorrhage. The rabbits show better peristalsis if the cord is destroyed from the lumbar to the midscapular region, but the dogs and cats do not seem to need such pithing. After applying the electrodes, the wound is closed as far as possible and moist gauze is used to protect the viscera from drying. In some experiments, and especially when working near the diaphragm, we have packed off with rubber dam so as to get rid of stray currents. The animals are kept warm on an electric pad. We hope later to study the action currents of the intact animal immersed in Locke's solution, when we can get a properly enameled tank. A metal tank with the usual thermo-regulator will not do on account of the large currents which are formed in the bath. The dogs have shown the most active gastric peristalsis and are probably most suitable for work on the stomach. Rabbits do very well for studies on the intestine.

Records are made by directing the beam of light reflected from the galvanometer mirrors through the slit of a camera on to a revolving

drum covered with bromide paper. In order to save space on the paper this camera is placed only 25 cm. from the mirrors. The time record is obtained by fastening a little mirror to the armature of a signal magnet so devised that the mirror will tilt when a current is sent in by way of the clock. The mechanical records are obtained by fastening a tiny mirror to one end of a straw about an inch long. The plane of the mirror is perpendicular to the long axis of the straw. The other end of the straw is fastened to the central point of the rubber membrane of an ordinary tambour. The straw passes through a hole in a little piece of stiff paper which is fastened to the edge of the tambour and serves as a fulcrum. When the membrane bulges, the mirror is tipped and the light spot moves on the drum. This tambour is connected to one of my enterographs, a simple little bellows which is fastened to the bowel by two tiny wire serrefines (6). As the whole apparatus is developing and changing from day to day as new needs arise and new knowledge is gained, further details will be given in a later paper. My object at present is simply to report progress and to point to a few things which are already clear.

Polarity of the bowel. It has been known for many years that there are gradients of electrical potential along the axes of plants and animals. The rapidly growing tip of a plant is negative to the trunk (2); the head of a hydroid polyp is negative to its stem, and anterior levels are negative to posterior ones (3). In recent years Child (4) and his students have found such electrical gradients in all the axiate animals and plants which they have studied. These gradients seem to be closely associated with, and due to, parallel ones of chemical activity or metabolism. As Miss Starkweather and I (5) have shown that there is a gradient of chemical activity in the bowel from duodenum to colon, I was much interested to see if a gradation in electrical potential could be demonstrated. Thus far considerable evidence has been obtained for such a view, but unfortunately much of the work done has not been entirely conclusive on account of the technical difficulties encountered. The muscle is in contact with the mucous membrane and this sometimes incloses masses of digesting food. Fortunately for us, the metabolic gradient in the mucous membrane seems to run quite parallel to that in the muscle. Otherwise the problem would be even more difficult. The chemical processes going on in the food seem to exert a big influence because in the rabbit the full cecum and stomach are generally markedly negative to all other parts of the tract. Another difficulty arises in the fact that the activity of the muscle varies from time to time in various

parts. Moreover, in order to compare the potential of the duodenum and cecum the bowel cannot remain in the body with the various coils in contact one with another, but it must be removed and so arranged that there are no short-circuits. That means trauma, a certain amount of drying, varying degrees of asphyxia, etc. Recently we have begun to get good results with freshly removed dogs' intestines stretched out in a long bath of warm Locke's solution. In some of these experiments there appeared a definite gradient of potential from the relatively negative duodenum to a point about a foot above the ileo-cecal sphincter. From that point the gradient rose again to the sphincter as we would expect from previous work on the rhythmic gradient (6). Similar gradients have been found in some rabbits, but so far we feel that the results are only suggestive. Further work will be reported later.

The character of the action currents. As will be seen from the records, the curves obtained in different regions with the galvanometer are remarkably similar to those traced by heart levers attached to bits of muscle from corresponding parts of the tract. Naturally, the records are simplified if the electrodes are kept close together on the gut. They are placed generally within a centimeter or two of each other so that the curves will not have too many components. The most interesting thing is that the mechanical and the electrical curves correspond not only as regards rate of contraction but also as regards amplitude and form. For instance, a glance at the illustrations in my fourth paper (7) will show that the contractions of strips of muscle removed from the lesser curvature of the stomach near the cardia are small and even and that their rate is fairly rapid. Figure 2*A* shows how closely the electrogastrogram from that region corresponds to the mechanogram. On the greater curvature the amplitude of contraction is larger, the waves uneven and the rate slower—the electrogram corresponds closely (fig. 2, *B* and *D*). In strips removed from the pyloric antrum the amplitude is very large, the peaks are sharp and symmetrical and the rate is slow, corresponding to that of the intact stomach. Again, the electrogram corresponds exactly (fig. 2, *C* and *F*). In the duodenum, the mechanogram shows small, even, and rapidly recurring waves—the electrogram has the same characteristics (figs. 3 and 4). The rate of rhythmic contraction varies inversely as the distance from the pylorus to the lower ileum. This rate in the intact animal is from 22 to 17 at the pylorus, to from 11 to 7 near the ileocecal sphincter. The rates obtained electrically are the same. The contractions obtained from segments of colon are generally slow and irregular and, again, the action

currents resemble them (fig. 9 *B*). This work brings, therefore, ample confirmation of that done before with mechanical records. It demonstrates clearly again the rhythmic gradient, and the marked differences in the types of activity shown by the muscle in the different parts of the tract.

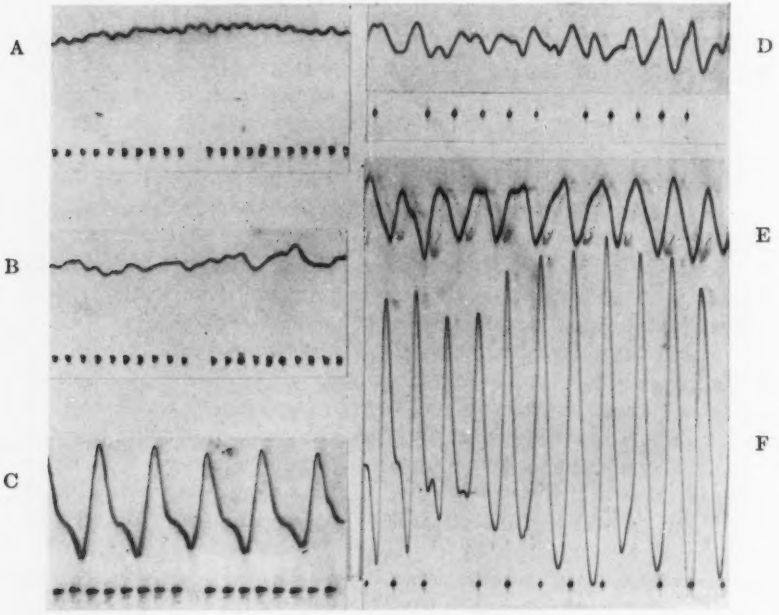

Fig. 2. To show action currents from different parts of the stomach: *A*, from the cardia of the cat; *B*, from the fundus of a cat; *C*, from the pyloric autrum of a cat; *D*, from the fundus of a dog; *E*, from the preantrum, and *F*, pyloric antrum of a dog. An upward deflection indicates negativity of the orad electrode.

The most remarkable thing about these action currents is that they are produced constantly in stomachs and intestines which, so far as the eye can detect, are absolutely motionless. Although, as we would expect, they are more powerful when the muscle is actively contracting, they are often just about as strong when no motion can be detected. Anyone who has done much work on the intestine with the abdomen open will realize what it means to have a technic which will enable him

to go right ahead with his experiment without waiting, perhaps hours, for the appearance of normal peristalsis As Auer particularly pointed out, when the abdomen is opened a hush seems to fall upon its contents. Stimuli coming through the splanchnic nerves cause an inhibition of the intestinal movements. To obviate that, it is customary to cut the splanchnic nerves or to destroy the lower part of the spinal cord.

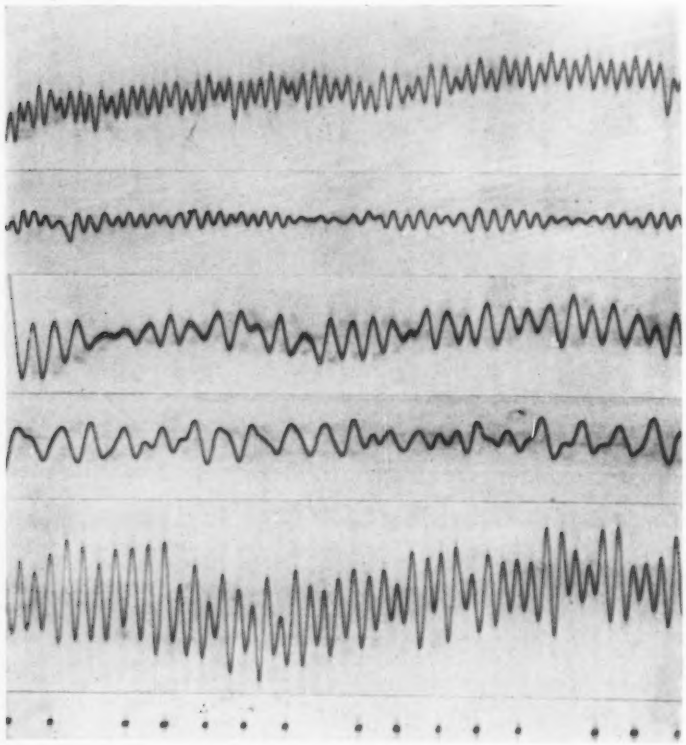

Fig. 3. To show the action currents and the rhythmic gradient in the rabbit's intestine from duodenum to ileum. The rates per minute from above downwards are: duodenum, 21.5; jejunum, 18.5; middle, 10.3; lower ileum, 7; terminal ileum, 15. The time record indicates 10-second intervals with a double gap every minute. Note irregularities due to the harmonic synthesis of components with slightly different rates. The "tone" changes in the terminal ileum come probably from the colon.

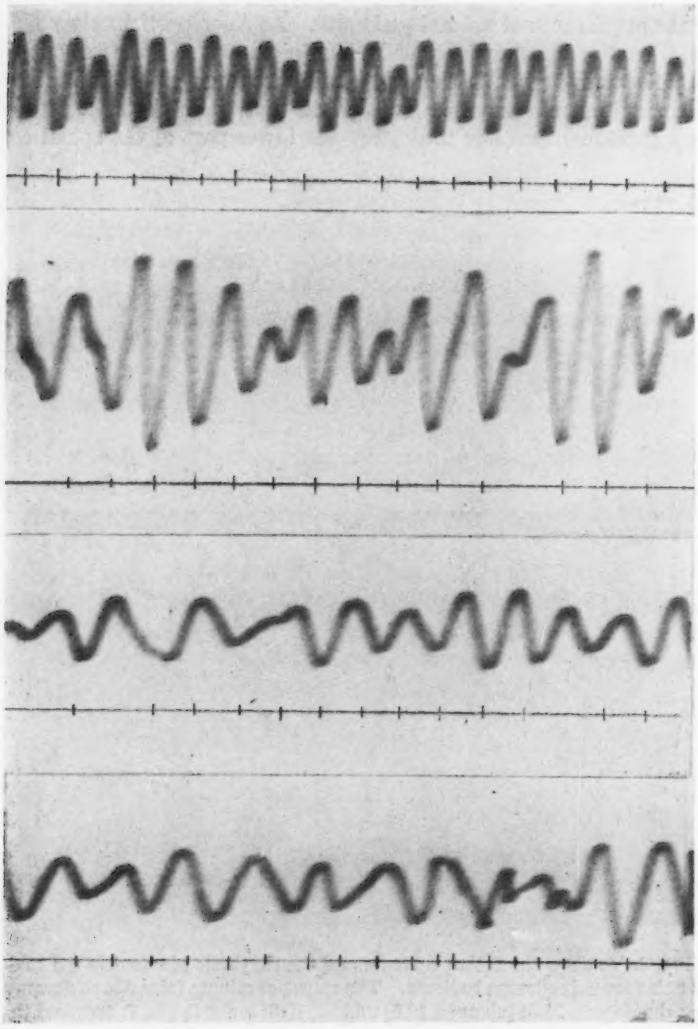

Fig. 4. To show the action currents and rhythmic gradient in the cat's intestine. The rates per minute from above downwards are: duodenum, 15.5; jejunum, 11; middle, 9; terminal ileum, 7. The time record indicates 5-second intervals with a double gap each minute.

Unfortunately, that does not always bring about an immediate return of peristalsis; and a large proportion of the animals so treated will not prove entirely satisfactory for experimental work. I have never been able to determine just why one animal will show good gastric peristalsis and ten others will not. Some workers get better results by giving castor oil, aloin or other drugs, but I have always hesitated to use such drugs before attempting "physiologic" experiments. The use of the ether or urethane is bad enough. Not infrequently, good peristalsis can be obtained only toward the end of a long experiment. After working for several years with such handicaps, it is a great pleasure to have a method which enables me to get good records as soon as the abdomen is opened. So far there have been no failures although, naturally, some animals produce more powerful currents than others.

The persistence of strong action currents in the presence of a quiet inhibited gut brings up some interesting problems. It would appear that the splanchnic nerves can block the mechanical contractions while, at the same time, they leave the rhythmic chemical processes which presumably give rise to the mechanical ones. It makes one think of an automobile with the engine running and the clutch out. From time to time some mechanism lets the "clutch" in, and motion appears. Stübel (8) and Marimon (9) have commented on this phenomenon. Stübel noticed in chickens that he could get good currents from absolutely quiet crops. Similar observations have been made also with the heart, where certain drugs or the absence of calcium will stop the contractions, but leave the action currents. Mines (10) believes that calcium helps in some way to convert the rhythmic chemical changes into mechanical contractions. The intestine offers a nice field for the study of this phenomenon. So far we have been able to show that vagus stimulation will produce temporary inhibition of the action currents followed by an increase in their amplitude. That response corresponds to the one observed at times in the mechanical record. Not infrequently faradic stimulation of the vagus strong enough markedly to affect the heart and lungs will have absolutely no effect on the electro-enterogram.

Problems in the stomach. An interesting problem has arisen in regard to the pacemaker of the stomach. As is well known, there are three waves of contraction to the minute in the stomach of man, and from two to five per minute in the laboratory animals. These waves appear, to the naked eye, to arise ordinarily about the middle of the stomach, but careful x-ray or mechanical records show that many of them develop

in ripples which come from the neighborhood of the cardia. As I was able to show that strips of muscle removed from the lesser curvature next to the cardia ordinarily have a faster rate of contraction than that of any other part of the stomach, I felt that that region is probably a sort of pace-maker. In the excised bits of muscle there is more or less gradation in rate from the cardia to the pylorus. At the cardia it is about 12 per minute; in the antrum it is about 3 per minute. The next question is: why should there be a discrepancy between the rates of the excised muscle and the intact organ? In the intestine there is practically no such difference. Why should a muscle which tends to beat 12 times per minute send out over the stomach only 3 waves per minute? The best answer seemed to be that the muscle in situ beats at a slower rate because that allows it more time for recovery and insures a higher efficiency. Now, to my surprise, I find that the cardiac muscle *in situ* gives signs of contracting rhythmically 8 or 12 times per minute, as will be seen in figure 5. In some rabbits the rate is around 20 per minute. The antrum, a few centimeters away, shows potential changes from 2 to 5 times a minute, and it can easily be shown that these correspond to the visible waves coursing over the stomach. If the rhythmic action currents detected near the cardia mean that the muscle there is pulsating 12 times a minute, the next question is, what relation do these pulsations bear to the waves which course over the stomach? Do the large waves originate in one of the small ones and if so, how does that particular one happen to gain the ascendancy? It would seem that with two galvanometers working together the matter could easily be settled, but many difficulties have arisen in the interpretation of the records. In many of them there is no difference in size in the cardiac oscillations and no sign of a rhythmic tonus change. Furthermore, the cardiac rate does not seem always to be an even or a constant multiple of the antral rate. In other records it seems quite certain that the waves coursing over the stomach are arising in tonus changes at the cardia; in still others it is hard to say whether or not the tonus waves are extrinsic and due simply to a spreading of the current from the antrum. We can see that there is some spreading because the cardiac records sometimes show respiratory oscillations, and the records from the pyloric antrum nearly always show oscillations corresponding to the duodenal contractions. It is possible, of course, that the cardiac region does actually pulsate at a rate faster than that of the antral end of the stomach. Basslinger, years ago, described such a pulsation; Palugyay has observed with the x-ray small waves running

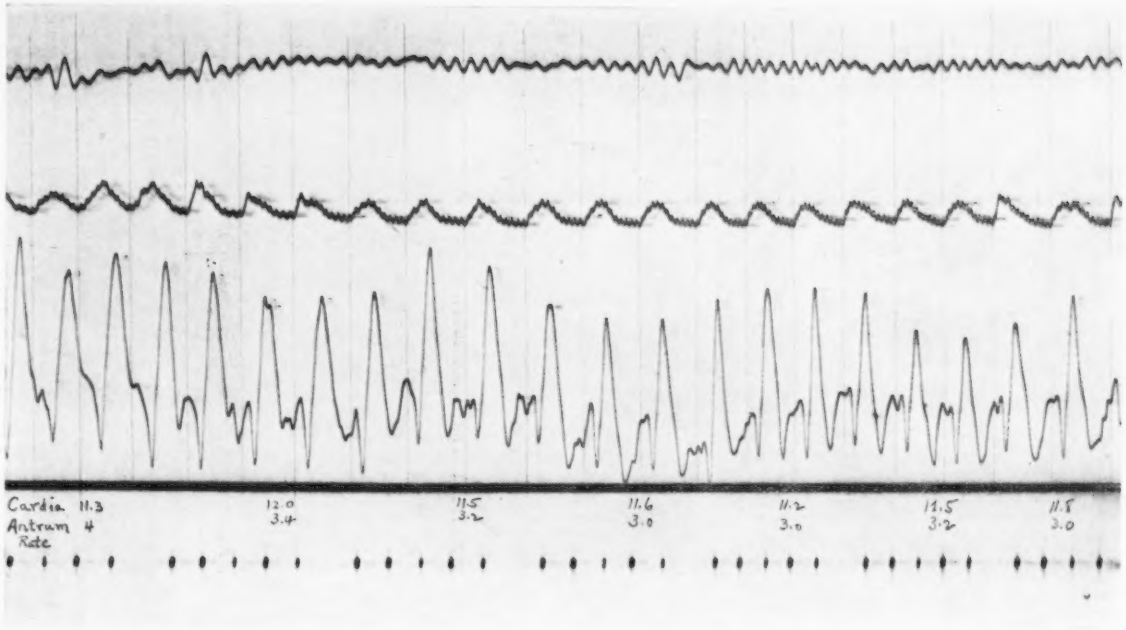

Fig. 5. From above downwards the records are: electrogram from the lesser curvature next to the cardia; mechanogram at the upper end of the pylorie antrum; electrogram from the pylorie antrum; signal magnet; time magnet at 10-second intervals. The animal was a large female dog with a poor intestinal gradient and a tendency to reverse peristalsis in the stomach. In this and all subsequent illustrations an upward deflection means that the cephalad electrode has become negative to the caudad one. The rate at the cardia varied between 11.2 and 12.5 per minute; at the antrum between 3 and 4 per minute. The smaller waves at the antrum have a rate between 19 and 24 per minute and may come from the duodenum. It will be noted that there is no constant relation between the rate at the cardia and that in the antrum. The smaller waves on the mechanogram are respiratory in origin; the large ones represent gastric peristalsis.

out from the cardia on to the fundus; waves different from those in the lower part of the stomach; and we have seen signs of such small frequent waves on some of our mechanical records. The fact that the frequent action currents from the cardia are detected also in excised

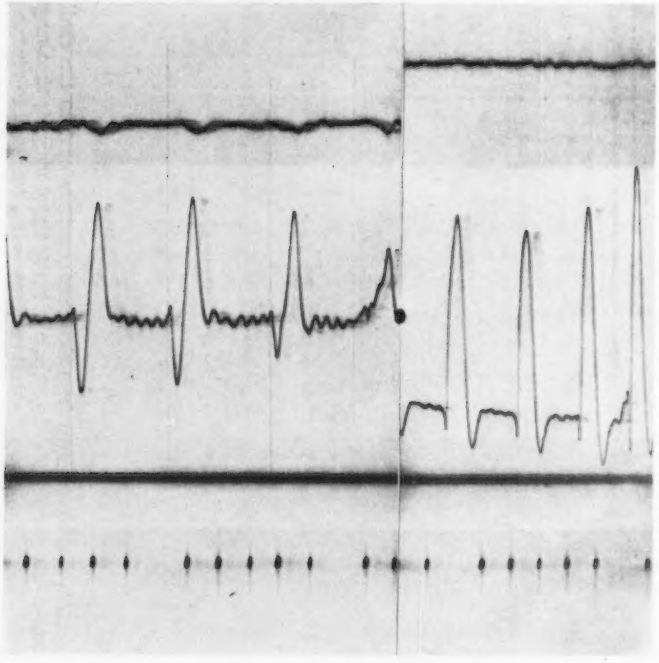

Fig. 6. To show shifting of the pace-maker. Electrograms on left from the lesser curvature near the cardia and preantrum on the greater curvature. The positive variation in the cardiac record and the predominately negative one in the preantral record indicate that the pace-maker is between them, sending waves in both directions. The records on the right taken a few minutes later from cardia and pyloric antrum show a different type of deflection. The animal was a dog. The tiny deflections on the cardiac record are respiratory—rate 36 per minute.

stomachs shows that they are intrinsic and not arising in the bowel or adjacent organs.

The negative (upward) variation seen in the figures shows that the waves are proceeding aborally over the stomach. Quite frequently,

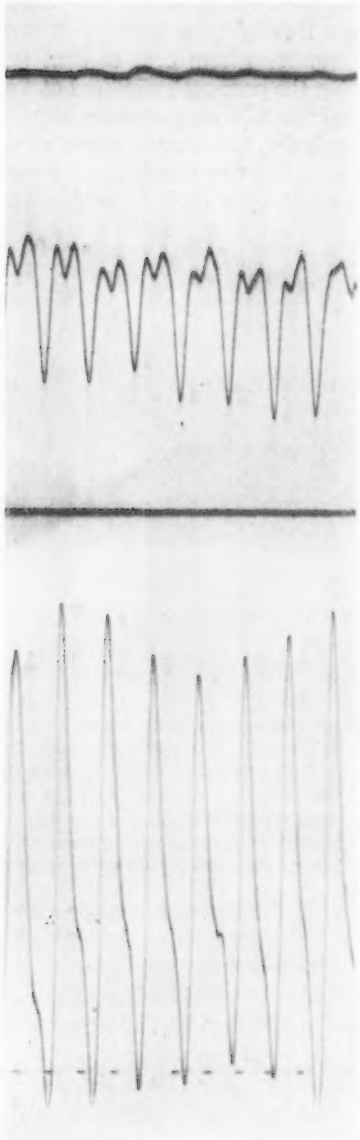

Fig. 7. To show again a positive variation in the cardia with a predominately negative variation in the preantral region. The uppermost record is a mechanogram from the fundus. The animal was a dog.

when the stomach is contracting slowly and irregularly; when the animal is sick, or when it is dying, it can be seen very clearly that the pace-maker shifts, sometimes from beat to beat (see figs. 6 and 7). In one distempered dog the pace-maker shifted at times to the pylorus and reverse waves could then be seen running over the stomach.

One difficulty in analyzing the records arises in the fact that ordinarily several waves are going over the stomach at one time. It is much simpler in the heart where each cycle is distinct and separate. For-

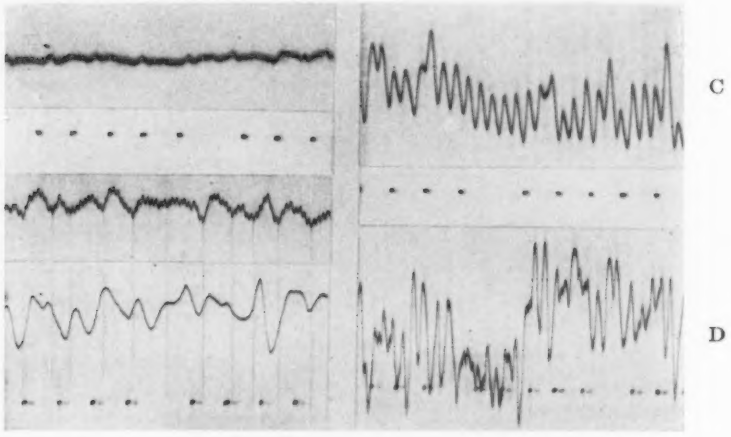

Fig. 8. To show: *A*, an electrogram from the gall bladder; *B*, electrograms from the liver and middle stomach; *C*, electrogram from the common duct; and *D*, electrogram from the first portion of the duodenum of a dog. The large deflections on the liver are generally derived from the stomach. In this case the small deflections are respiratory in origin, but they often come from the duodenum. The deflections in the common duct may also come from the duodenum but I am not yet sure of it, as the rates do not agree exactly.

tunately, irregularities in the interval between waves often enable us to identify corresponding deflections at the cardia and at the pylorus.

Other observations. This technic may be very helpful in studying the motility of the gall bladder and bile ducts although precautions will have to be taken to make sure that the deflections obtained are intrinsic and not derived from the adjacent stomach or bowel. Figure 8 shows a characteristic gall-bladder record from the dog. The contractions seem ordinarily to be derived from the adjacent duodenum but in some

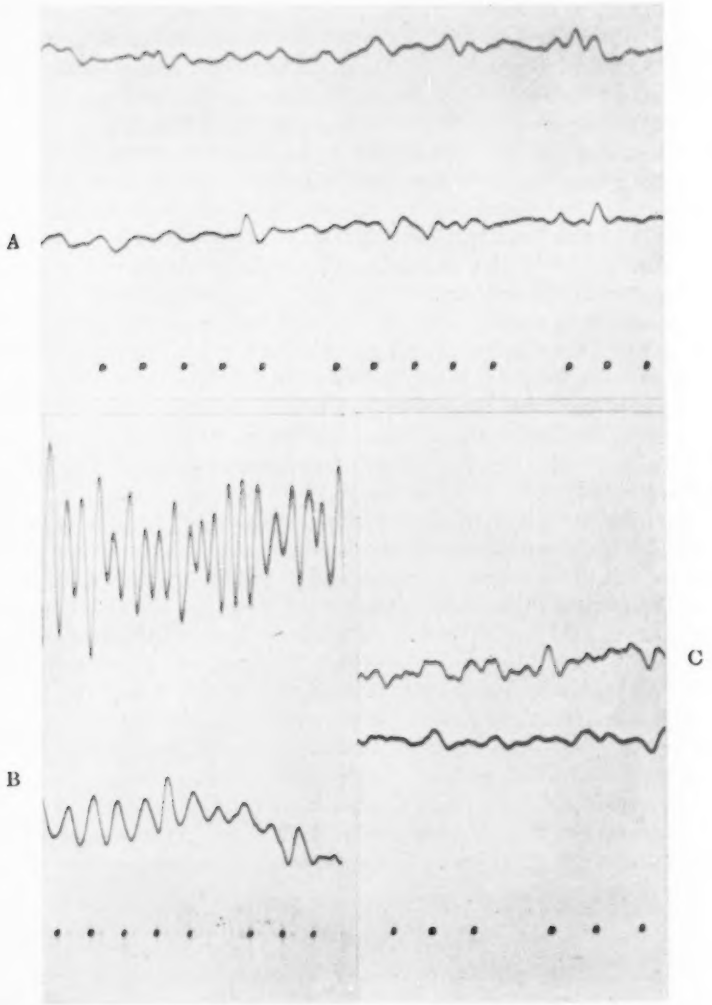

Fig. 9. Showing electrograms from *A*, the orad and middle regions of the rabbit's cecum; *B*, the terminal ileum and colon of a dog; and *C*, the colon and rectum of a rabbit. The time record represents 10-second intervals with a double gap every minute.

cases another, probably intrinsic, rhythm appears. The next curve was obtained from the liver and consists of deflections derived from diaphragm and stomach. The next curve is from the common duct. The rate of contraction is that usually found in the duodenum, but the accompanying record which was taken a moment later from the upper duodenum of the same animal has a very different appearance. The rabbit's cecum (fig. 9) shows small waves, derived perhaps from the ileum, with larger contractions superimposed once every minute or so.

It has always been supposed that there is little connection between the movements of the stomach and duodenum because the gastric waves stop at the pylorus. Many years ago I obtained considerable evidence which made me think that the gastric waves influence the activities of the duodenum and give rise to the rush waves which run from time to time down the length of the bowel. The new technic practically confirms these suspicions and shows marked electrical changes in the duodenum following the arrival of gastric waves in the pyloric antrum. The matter will be discussed more at length in a subsequent paper.

The demonstration of these electric currents, spreading from one part of the digestive tract to another and from the tract to adjacent glands like the liver, brings up some interesting problems. If Ralph Lillie and others are correct in their belief that the bio-electric currents have much to do with the institution and direction of chemical activities in the body it seems likely that these spreading abdominal currents might have something to do with that correlation of mechanical and chemical functions which is often attributed to hormones and nerves. At any rate, the subject would seem to be worth looking into a little farther.

We have done some work, stimulating the bowel between two pairs of electrodes, with the hope of demonstrating the "law of the intestine," but so far without success. These experiments will be continued and reported upon later.

SUMMARY

New electrodes and other apparatus have been devised, by means of which the action currents of the digestive tract can easily be studied. Two electro-enterograms and two mechanograms are recorded on the same drum.

Some evidence has been found of gradients of potential along the stomach from cardia to pylorus and along the bowel from duodenum

to colon. Orad regions are negative to caudad ones. In the rabbit, the stomach and cecum, full of digesting food, are negative to other parts of the tract.

The electro-enterograms obtained by attaching electrodes to various parts of the stomach and bowel resemble closely the mechanograms of the rhythmic contractions of bits of muscle excised from those parts.

The rates of rhythmic contraction correspond in the two types of records; and the rhythmic gradients in stomach and bowel have been demonstrated clearly again.

It is a striking fact that large currents are obtained from stomachs and bowels which, to the naked eye, show absolutely no peristaltic movement. Splanchnic stimuli which inhibit the contractions of the bowel have little or no effect on the rhythmic action currents.

In the stomach a negative variation travels from the cardiac region to the pylorus every 12 to 30 seconds. The cardiac region shows from 6 to 20 deflections a minute, superimposed sometimes upon other large tone-like changes which are synchronous with the gastric waves. The antral end of the stomach shows ordinarily only the deflections corresponding to the visible waves. Often these deflections are superimposed upon other extrinsic ones from the duodenum.

It is yet a question why only three waves a minute arise in a region which appears to be contracting from 6 to 20 times a minute.

At times it can be shown that the pace-maker shifts, even to the pyloric region.

Records are shown from the gall bladder, liver, common bile duct and cecum.

It has been found that there are marked electrical changes in the duodenum following the arrival of gastric waves at the pylorus.

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A REVOLVING CONTACT-MAKER TO STIMULATE AT ANY REQUIRED FREQUENCY

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The ordinary device used for showing summation of stimuli, namely, a vibrating spring of variable length with a pointer moving in and out of a bowl of nercury, is clumsy and unsatisfactory for ordinary class work as it requires certain precautions in use and is apt to lead to wastage of mercury. To avoid the difficulties arising with this instrument a simple revolving contact-maker has been devised, as shown in figure 1, which consists of a circular plate of vulcanite with a number of phosphorbronze strips inlet into its circumference flush with the surface, on which a phosphorbronze or stencil copper spring rubs as it revolves and so makes a number of short bursts of current which may be used for stimulation. The stand of the instrument consists of a heavy casting, and the vulcanite plate is mounted upon a spindle driven by a cone-pulley from a separate motor or from the shafting driving the drum, or from one of the pulleys on the drum itself. The battery is connected through the contact-maker to a pair of electrodes, which are used for stimulation of the tissue, so that each time a strip of phosphor-bronze touches the spring a current momentarily passes through the tissue and gives it a shock. The instrument can be driven at any required speed, and the form of the current given by it as it revolves is very regular, as shown in the lower records of figure 2, which were taken by connecting the instrument through the copper string in a string galvanometer. Typical records made with a frog's muscle are shown in the upper curves of figure 2. The instrument can of course be used with an nduction coil, being connected through the primary in the usual way, and the stimulating current taken from the secondary; it is on the whole however more satisfactory if used without an induction coil and directly connected to the electrodes. It is a simple and cheap instrument to construct, and in addition to being useful for class purposes it may prove valuable for any experiment in which a series of shocks at any desired frequency is required. Its design is due largely to Mr. A. C. Downing of this Department.

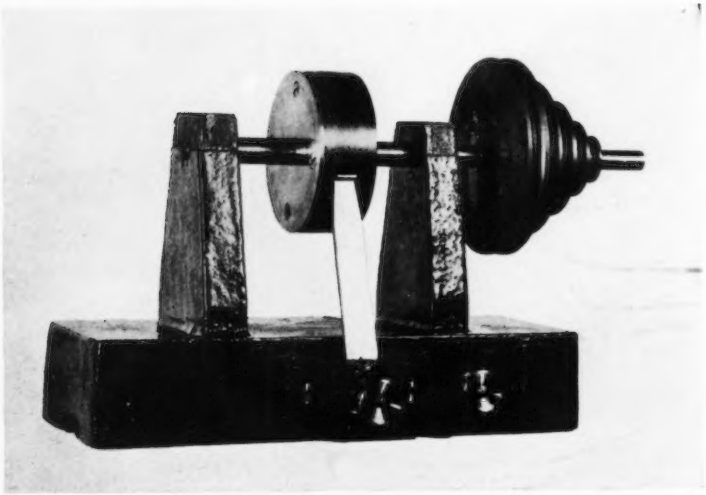

Fig. 1. Revolving contact-maker. Note the strip of phosphor-bronze in the vulcanite just above the spring.

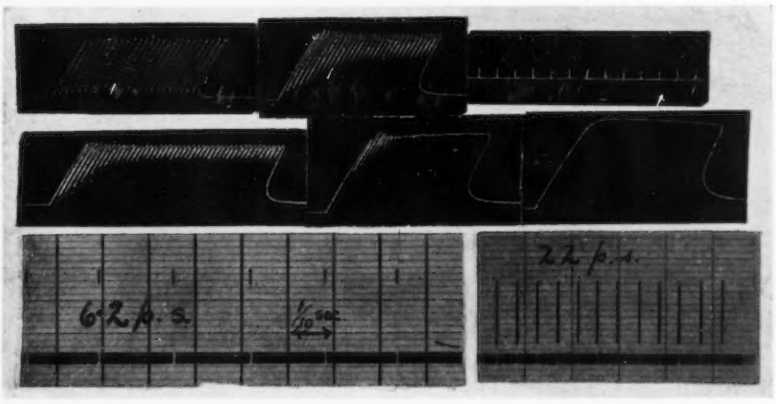

Fig. 2. *Upper records:* isotonic contraction of frog's gastrocnemius stimulated at 5 different frequencies at 10°C. Right hand top curve, time marker, seconds.
Lower records: Stimulating currents from contact-maker, at 6.2 and 22 contacts per second respectively, put through copper string of string galvanometer. Time in $\frac{1}{10}$ second.